

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

66/67/50
U.S. PTO**UTILITY****PATENT APPLICATION
TRANSMITTAL**

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No. REG 203-A

First Inventor or Application Identifier Neil Stahl

Title Receptor Based Antagonists, and Methods
of Making and Using

Express Mail Label No. EJ 468 542 323 US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. * Fee Transmittal Form (e.g., PTO/SB/17)
(Submit an original and a duplicate for fee processing)

2. Specification [Total Pages 69]

- Descriptive title of the Invention
- Cross References to Related Applications
- Statement Regarding Fed sponsored R & D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings (if filed)
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. Drawing(s) (35 U.S.C. 113) [Total Sheets 69]

4. Oath or Declaration [Total Pages]

- a. Newly executed (original or copy)
- b. Copy from a prior application (37 C.F.R. § 1.63(d))
(for continuation/divisional with Box 16 completed)
- i. DELETION OF INVENTOR(S)
Signed statement attached deleting
inventor(s) named in the prior application,
see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

*NOTE FOR ITEMS 1 & 13: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).

ADDRESS TO: Assistant Commissioner for Patents
Box Patent Application
Washington, DC 20231

5. Microfiche Computer Program (Appendix)
6. Nucleotide and/or Amino Acid Sequence Submission
(if applicable, all necessary)
- a. Computer Readable Copy
- b. Paper Copy (identical to computer copy)
- c. Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. Assignment Papers (cover sheet & document(s))
8. 37 C.F.R. § 3.73(b) Statement Power of
(when there is an assignee) Attorney
9. English Translation Document (if applicable)
10. Information Disclosure Statement (IDS)/PTO-1449 Copies of IDS
Statement (IDS)/PTO-1449 Citations
11. Preliminary Amendment
12. Return Receipt Postcard (MPEP 503)
(Should be specifically itemized)
13. * Small Entity Statement(s) Statement filed in prior application,
(PTO/SB/09-12) Status still proper and desired
14. Certified Copy of Priority Document(s)
(if foreign priority is claimed)
15. Other: unexecuted...Declaration...and
Power of Attorney.....

16. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment

Continuation Divisional Continuation-in-part (CIP) of prior application No: _____ / _____

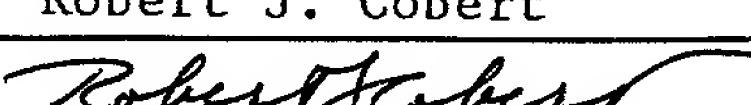
Prior application information: Examiner _____

Group / Art Unit: _____

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

17. CORRESPONDENCE ADDRESS

<input type="checkbox"/> Customer Number or Bar Code Label	(Insert Customer No. or Attach bar code label here)			or <input type="checkbox"/> Correspondence address below
Name	Robert J. Cobert			
	Regeneron Pharmaceuticals, Inc.			
Address	777 Old Saw Mill River Road			
City	Tarrytown	State	New York	Zip Code
Country	United States	Telephone	914-345-7400	Fax

Name (Print/Type)	Robert J. Cobert	Registration No (Attorney/Agent)	36,108
Signature		Date	May 19, 1999

Burden Hour Statement This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

RECEPTOR BASED ANTAGONISTS AND
METHODS OF MAKING AND USING

This application claims priority of U.S. Provisional application No. 60/101,858
5 filed September 25, 1998. Throughout this application various publications
are referenced. The disclosures of these publications in their entireties are
hereby incorporated by reference into this application.

BACKGROUND OF THE INVENTION

10

Although discovered for varying biological activities, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM) and interleukin-6 (IL-6) comprise a defined family of cytokines (referred to herein as the "CNTF family" of cytokines). These cytokines are grouped together
15 because of their distant structural similarities [Bazan, J. Neuron 7: 197-208 (1991); Rose and Bruce, Proc. Natl. Acad. Sci. USA 88: 8641-8645 (1991)], and, perhaps more importantly, because they share " β " signal-transducing receptor components [Baumann, et al., J. Biol. Chem. 265:19853-19862 (1993); Davis, et al., Science 260: 1805-1808 (1993); Gearing et al., Science 255:1434-1437 (1992); Ip
20 et al., Cell 69: 1121-1132 (1992); Stahl, et al., J. Biol. Chem. 268: 7628-7631 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Receptor activation by this family of cytokines results from either homo- or hetero-dimerization of these β components [Davis, et al. Science 260: 1805-1808 (1993), Murakami, et al., Science 260: 1808-1810 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)].
25 IL-6 receptor activation requires homodimerization of gp130 [Murakami, et al. Science 260: 1808-1810 (1993), Hibi, et al., Cell 63: 1149-1157 (1990)], a protein initially identified as the IL-6 signal transducer [Hibi, et al., Cell 63: 1149-1157

(1990)]. CNTF, LIF and OSM receptor activation results from heterodimerization between gp130 and a second gp130-related protein known as LIFR β [Davis, et al., Science 260: 1805-1808 (1993)], that was initially identified by its ability to bind LIF [Gearing et al., EMBO J. 10: 2839-2848 (1991)].

5

In addition to the β components, some of these cytokines also require specificity-determining " α " components that are more limited in their tissue distribution than the β components, and thus determine the cellular targets of the particular cytokines [Stahl and Yancopoulos, Cell 74: 587-590 (1993)].

10 Thus, LIF and OSM are broadly acting factors that may only require the presence of gp130 and LIFR β on responding cells, while CNTF requires CNTFR α [Stahl and Yancopoulos, Cell 74: 587-590 (1993)] and IL-6 requires IL-6R α [Kishimoto, et al., Science 258: 593-597 (1992)]. Both CNTFR α (Davis et al., Science 259:1736-1739 (1993) and IL-6R α [Hibi, et al. Cell 63:1149-1157, 15 Murakami, et al., Science 260:1808-1810 (1990); Taga, et al., Cell 58:573-581 (1989)] can function as soluble proteins, consistent with the notion that they do not interact with intracellular signaling molecules but that they serve to help their ligands interact with the appropriate signal transducing β subunits [Stahl and Yancopoulos, Cell 74: 587-590 (1993)].

20

Additional evidence from other cytokine systems also supports the notion that dimerization provides a common mechanism by which all cytokine receptors initiate signal transduction. Growth hormone (GH) serves as perhaps the best example in this regard. Crystallographic studies have 25 revealed that each GH molecule contains two distinct receptor binding sites, both of which are recognized by the same binding domain in the receptor, allowing a single molecule of GH to engage two receptor molecules [de Vos, et

al., Science 255: 306-312 (1992)]. Dimerization occurs sequentially, with site 1 on the GH first binding to one receptor molecule, followed by the binding of site 2 to a second receptor molecule [Fuh, et al., Science 256: 1677-1680 (1992)].

Studies with the erythropoietin (EPO) receptor are also consistent with the

- 5 importance of dimerization in receptor activation, as EPO receptors can be constitutively activated by a single amino acid change that introduces a cysteine residue and results in disulfide-linked homodimers [Watowich, et al., Proc. Natl. Acad. Sci. USA 89:2140-2144 (1992)].

- 10 In addition to homo- or hetero-dimerization of β subunits as the critical step for receptor activation, a second important feature is that formation of the final receptor complex by the CNTF family of cytokines occurs through a mechanism whereby the ligand successively binds to receptor components in an ordered manner [Davis, et al. Science 260:1805-1818 (1993); Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Thus CNTF first binds to CNTFR α , forming a complex which then binds gp130 to form an intermediate (called here the $\alpha\beta_1$ intermediate) that is not signaling competent because it has only a single β component, before finally recruiting LIFR β to form a heterodimer of β components which then initiates signal transduction. Although a
- 15 similar intermediate containing IL-6 bound to IL-6R α and a single molecule of gp130 has not been directly isolated, we have postulated that it does exist by analogy to its distant relative, CNTF, as well as the fact that the final active IL-6 receptor complex recruits two gp130 monomers. Altogether, these findings led to a proposal for the structure of a generic cytokine receptor complex
- 20 (Figure 1) in which each cytokine can have up to 3 receptor binding sites: a site that binds to an optional α specificity-determining component (α site), a site that binds to the first β signal-transducing component (β_1 site), and a site
- 25

that binds to the second β signal-transducing component (β_2 site) [Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. These 3 sites are used in sequential fashion, with the last step in complex formation -- resulting in β component dimerization -- critical for initiating signal transduction [Davis, et al. Science 5 260:1805-1818 (1993)]. Knowledge of the details of receptor activation and the existence of the non-functional β_1 intermediate for CNTF has led to the finding that CNTF is a high affinity antagonist for IL-6 under certain circumstances, and provides the strategic basis for designing ligand or receptor-based antagonists for the CNTF family of cytokines as detailed below.

10

Once cytokine binding induces receptor complex formation, the dimerization of β components activates intracellular tyrosine kinase activity that results in phosphorylation of a wide variety of substrates [Ip, et al. Cell 69:121-1132

(1992)]. This activation of tyrosine kinase appears to be critical for

15

downstream events since inhibitors that block the tyrosine phosphorylations also prevent later events such as gene inductions [Ip, et al., Cell 69:121-1132 (1992); Nakajima and Wall, Mol. Cell. Biol. 11:1409-1418 (1991)]. Recently, we have demonstrated that a newly discovered family of non-receptor tyrosine kinases that includes Jak1, Jak2, and Tyk2 (referred to as the Jak/Tyk kinases)

20

[Firnbach-Kraft, et al., Oncogene 5:1329-1336 (1990); Wilks, et al., Mol. Cell. Biol. 11: 2057-2065 (1991] and that are involved in signal transduction with other cytokines [Argetsinger, et al., Cell 74:237-244 (1993); Silvennoinen, et al., Proc. Natl. Acad. Sci. USA 90:8429-8433 (1993); Velazquez, et al., Cell 70: 313-322 (1992); Witthuhn, et al., Cell 74:227-236 (1993)], preassociate with the

25

cytoplasmic domains of the β subunits gp130 and LIFR β in the absence of ligand, and become tyrosine phosphorylated and activated upon ligand addition [Stahl et al., Science 263:92-95 (1994)]. Therefore these kinases appear

to be the most proximal step of intracellular signal transduction activated inside the cell as a result of ligand binding outside of the cell. Assay systems for screening collections of small molecules for specific agonist or antagonist activities based on this system are described below.

5

The CNTF family of cytokines play important roles in a wide variety of physiological processes that provide potential therapeutic applications for both antagonists and agonists.

10

SUMMARY OF THE INVENTION

An object of the present invention is the production of cytokine antagonists that are useful in the treatment of cytokine related diseases or disorders.

15

Another object of the invention is the use of the disclosed cytokine antagonists for the treatment of cytokine related diseases or disorders. For example, an IL-6 antagonist described herein may be used for the treatment of osteoporosis, the primary and second effects of cancers, including multiple myeloma, or cachexia.

20

Another object of the invention is the development of screening systems useful for identifying novel agonists and antagonists of cytokine receptors.

Another object of the invention is the development of screening systems useful for identifying small molecules that act as agonists or antagonists of

25 the cytokines.

Another object of the invention is the development of screening systems

useful for identifying novel agonists and antagonists of members of the CNTF family of cytokines.

Another object of the invention is the development of screening systems
5 useful for identifying small molecules that act as agonists or antagonists of the CNTF family of cytokines.

BRIEF DESCRIPTION OF THE FIGURES

10

FIGURE 1: Ordered binding of receptor components in a model of a generic cytokine receptor. The model indicates that cytokines contain up to 3 receptor binding sites and interact with their receptor components by binding first the optional α component, followed by binding to β_1 , and then β_2 . The β components for many cytokine receptors interact through membrane proximal regions (shaded boxes) with the Jak/Tyk family of cytoplasmic protein tyrosine kinases. Only upon dimerization of β components is signal transduction initiated, as schematized by the tyrosine phosphorylations (P) of the β components and the Jak/Tyk kinases.

20

FIGURE 2: CNTF inhibits IL-6 responses in a PC12 cell line (called PC12D) that expresses IL6R α , gp130, CNTFR α , but not LIFR β . Serum-deprived PC12D cells were incubated + IL-6 (50 ng/mL) in the presence or absence of CNTF as indicated. Some plates also received soluble IL6R α (1 mg/mL) or soluble CNTFR α (1 mg/mL) as indicated. Cell lysates were subjected to immunoprecipitation with anti-gp130 and immunoblotted with anti-phosphotyrosine. Tyrosine phosphorylation of gp130 is indicative of IL-6

induced activation of the IL-6 receptor system, which is blocked upon coaddition of CNTF.

FIGURE 3: Scatchard analysis of iodinated CNTF binding on PC12D cells.

5 PC12D cells were incubated with various concentrations of iodinated CNTF in the presence or absence of excess non-radioactive competitor to determine the specific binding. The figure shows a Scatchard plot of the amount of iodinated CNTF specifically bound, and gives data consistent with two binding sites with dissociation constants of 9 pM and 3.4 nM.

10

FIGURE 4. The amino acid sequence of human gp130-Fc-His₆. Amino acids 1 to 619 are from human gp130 (Hibi et al., Cell 63:1149-1157 (1990). Note that amino acid number 2 has been changed from a Leu to a Val in order to accommodate a Kozak sequence in the coding DNA sequence. The signal 15 peptide of gp130-Fc-His₆ has been italicized (amino acids 1 to 22). The Ser-Gly bridge is shown in bold type (amino acids 620, 621). Amino acids 662 to 853 are from the Fc domain of human IgG1 (Lewis, et al., J. Immunol. 151:2829-2838 (1993). (†) mark the two cysteines (amino acids number 632 and 635) of the IgG hinge preceding the Fc that form the inter-chain disulfide bridges that 20 link two Fc domains. The hexahistidine tag is shown in bold/italic type (amino acids 854 to 859). (•) shows the position of the STOP codon.

FIGURE 5. The amino acid sequence of human IL-6R α -Fc. Key: Amino acids 1 to 358 are from human IL-6R α (Yamasaki, et al., Science 241:825-828 (1988). 25 Note that amino acid number 2 has been changed from a Leu to a Val in order to accommodate a Kozak sequence in the coding DNA sequence. The signal peptide of IL-6R α -Fc has been italicized (amino acids 1 to 19). The Ala-

Gly bridge is shown in bold type (amino acids 359, 360). Amino acids 361 to 592 are from the Fc domain of human IgG1 (Lewis et al., J. Immunol. 151:2829-2838 (1993). (†) mark the two cysteines (amino acids number 371 and 374) of the IgG hinge preceding the Fc that form the inter-chain disulfide
5 bridges that link two Fc domains. (•) shows the position of the STOP codon.

FIGURE 6: The CNTF/IL-6/IL-11 receptor system. The ordered formation of the hexameric signal transducing receptor complex is depicted schematically. The cytokine associates with the R α component to form an obligatory

10 cytokine•R α complex (K_d is about 5 nM). This low affinity complex next associates with the first signal transducing component, marked β_1 , to form a high affinity cytokine•R α • β_1 complex (K_d is about 10 pM). In the case of IL-6R α , this component is gp130. This trimeric high affinity complex subsequently associates with another such complex. Formation of this
15 complex results in signal transduction as it involves dimerization of two signal transducing components, marked β_1 and β_2 respectively (adapted from (Ward et al., J. Bio. Chem. 269:23286-23289 (1994); Stahl and Yancopoulos, J. Neurobiology 25:1454-1466 (1994); Stahl and Yancopoulos, Cell 74:587-590
20 (1993).

FIGURE 7: Design of heterodimeric receptor-based ligand traps for IL-6.

The heterodimeric ligand trap is comprised of two interdisulfide linked proteins, gp130-Fc and IL-6R α -Fc. The gp130-Fc•IL-6R α -Fc complex (upper panel) is shown to mimic the high affinity cytokine•R α • β_1 complex (lower panel). The ligand trap functions as an antagonist by sequestering IL-6 and thus rendering unavailable to interact with the native receptors on IL-6-responsive cells.

FIGURE 8. Heteromeric immunoglobulin Heavy/Light Chain Receptor Fusions. An example of a heavy/light chain receptor fusion molecule is schematically depicted. The extracellular domain of gp130 is fused to C γ , whereas the extracellular domain of IL-6R α is fused to the constant region of the kappa chain (κ). The inter-chain disulfide bridges are also depicted (S-S).

5 FIGURE 9. Amino acid sequence of gp130-C γ 1. Key: Amino acids 1 to 619 are from human gp130 (Hibi, et al., Cell 63:1149-1157 (1990). Ser-Gly bridge is shown in bold type. Amino acids 662 to 651 are from the constant region of 10 human IgG1 (Lewis et al., J. Immunol. 151:2829-2838 (1993). (*) shows the position of the STOP codon.

15 FIGURE 10. Amino acid sequence of gp130 Δ 3fibro. Key: Amino acids 1 to 330 are from human gp130 (Hibi et al., Cell 63:1149-1157 (1990). Other symbols as described in Figure 9.

20 FIGURE 11. Amino acid sequence of J-CH1. Key: The Ser-Gly bridge is shown in bold, the J-peptide is shown in italics, the CH1 domain is underlined.

FIGURE 12. Amino acid sequence of C γ 4. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 239 comprise the C γ 4 sequence.

25 FIGURE 13. Amino acid sequence of κ -domain. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 108 comprise the κ domain. The C-terminal cysteine (amino acid 108) is involved in the disulfide bond of the κ domain with the CH1 domain of C γ .

FIGURE 14. Amino acid sequence of λ -domain. Key: The Ser-Gly bridge is shown in bold type. Amino acids 2 to 106 comprise the λ domain (Cheung, et al., J. Virol. 66: 6714-6720 (1992). The C-terminal cysteine (amino acid 106) is involved in the disulfide bond of the λ domain with the CH1 domain of Cy.

FIGURE 15. Amino acid sequence of the soluble IL-6R α domain. Key: Amino acids 1 to 358 comprise the soluble IL-6R α domain (Yamasaki, et al., Science 241:825-828 (1988). The Ala-Gly bridge is shown in bold type.

FIGURE 16. Amino acid sequence of the soluble IL-6R α 313 domain: Key: Amino acids 1 to 313 comprise the truncated IL-6R α domain (IL-6R α 313). The Thr-Gly bridge is shown in bold type.

FIGURE 17: Purification of gp130-Cy1•IL-6R α - κ . 4% to 12% SDS-PAGE gradient gel run under non-reducing conditions. Proteins were visualized by staining with silver. Lane 1: approximately 100 ng of material purified over Protein A Sepharose (Pharmacia). Lane 2: Molecular size standards (Amersham). Lane 3: The Protein A-purified material shown here after further purification over an IL-6 affinity chromatography step. The positions of the gp130-Cy1 dimer [(gp130-Cy1)₂], the gp130-Cy1 dimer associated with one IL-6R α - κ [(gp130-Cy1)₂•(IL-6R α - κ)₁], and the gp130-Cy1 dimer associated with two IL-6R α - κ [(gp130-Cy1)₂•(IL-6R α - κ)₂] are shown, as well as the sizes for the molecular size standards in kilodaltons (200, 100, and 46).

FIGURE 18. IL-6 dissociates slowly from the ligand trap. The dissociation

rate of IL-6 from a heavy/light chain receptor-based ligand trap (gp130-C γ 1•IL-6R α - κ) was compared to that obtained with the neutralizing monoclonal antibody B-E8 (BE8 MAb).

5 FIGURE 19. IL-6 can induce multimerization of the ligand trap. (A) Two different ligand traps are depicted schematically and listed according to their ability to bind protein A. gp130-Fc•IL-6R α -Fc (GF6F) binds protein A via its Fc-domains, whereas gp130-CH1•IL-6R α - κ (G16K) does not bind to protein A.

10 (B) Anti-kappa western blotting of proteins precipitated with Protein A-Sepharose from mixtures of GF6F ± IL-6, G16K ± IL-6, or GF6F plus G16K ± IL-6, as marked.

FIGURE 20. Inhibition of IL-6-dependent XG-1 cell proliferation. XG-1 cells [Zhang, et al., Blood 83:3654-3663 (1994)] were prepared for a proliferation assay by starving the cells from IL-6 for 5 hours. Assays were set up in 96-well tissue culture dishes in RPMI + 10% fetal calf serum + penicillin/streptomycin + 0.050 nM 2-mercaptoethanol + glutamine. 0.1 ml of that media was used per well. Cells were suspended at a density of 250,000 per ml at the start of the assay. 72 hours post addition of IL-6 ± ligands traps or antibodies, an MTT assay was performed as described (Panayotatos et al. Biochemistry 33:5813-5818 (1994). The different ligand traps utilized are listed.

20 FIGURES 21A-21D - Nucleotide sequence encoding and deduced amino acid sequence of fusion polypeptide designated 424 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

FIGURES 22A-22D - Nucleotide sequence encoding and deduced amino acid

sequence of fusion polypeptide designated 603 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

FIGURES 23A-23D - Nucleotide sequence encoding and deduced amino acid

5 sequence of fusion polypeptide designated 622 which is capable of binding the cytokine IL-4 to form a nonfunctional complex.

FIGURE 24A-24F - Nucleotide sequence encoding and deduced amino acid

sequence of fusion polypeptide designated 412 which is capable of binding the

10 cytokine IL-6 to form a nonfunctional complex.

FIGURE 25A-25F - Nucleotide sequence encoding and deduced amino acid

sequence of fusion polypeptide designated 616 which is capable of binding the

cytokine IL-6 to form a nonfunctional complex.

15

FIGURE 26A-26E - Nucleotide sequence encoding and deduced amino acid

sequence of fusion polypeptide designated 569 which is capable of binding the

cytokine IL-1 to form a nonfunctional complex.

20

FIGURE 27 - shows that an IL-4 trap designated 4SC375, which is a fusion

polypeptide of IL-2R γ -scb-IL4R α -Fc Δ C1, is several orders of magnitude better

as an IL-4 antagonist than IL4R α Fc Δ C1 alone in the TF1 cell bioassay.

FIGURE 28 - shows that an IL-4 trap designated 4SC375 displays antagonistic

25

activity in the TF1 cell bioassay equivalent to an IL-4 trap designated 4SC424

(described in Figs. 21A-21D) which is a fusion polypeptide of IL-2R γ -IL4R α -

Fc Δ C1 having the IL-2R γ component flush with the IL-4R α component.

FIGURE 29 - shows that the IL6 trap (6SC412 IL6R-scb-gpx-Fc Δ C1) described in Figs. 24A-24F is a better antagonist of IL-6 in the XG1 bioassay than the neutralizing monoclonal antibody to human IL-6 - BE8.

5

FIGURE 30 - shows that the trap 1SC569 (described in Figs. 26A-26E) is able to antagonize the effects of IL-1 and block the IL-6 production from MRC 5 cells upon treatment with IL-1.

10 FIGURE 31A-31G - The nucleotide and encoded amino acid sequence of the IL-4R α .IL-13R α 1.Fc single chain trap construct is set forth.

FIGURE 32A-32G - The nucleotide and encoded amino acid sequence of the IL-13R α 1.IL-4R α .Fc single chain trap construct is set forth.

15

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising:

- a) a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor;
- b) a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and
- c) a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

By "cytokine binding portion" what is meant is the minimal portion of the extracellular domain necessary to bind the cytokine. It is accepted by those of skill in the art that a defining characteristic of a cytokine receptor is the

- 5 presence of the two fibronectin-like domains that contain canonical cysteines and of the WSXWS box (Bazan, J.F., 1990, PNAS 87: 6934-6938). Sequences encoding the extracellular domains of the binding component of the cytokine's receptor and of the signal transducing component of the cytokine's receptor may also be used to create the fusion polypeptide of the invention.
- 10 Similarly, longer sequences encoding larger portions of the components of the cytokine's receptor may be used. However, it is contemplated that fragments smaller than the extracellular domain will function to bind the cytokine and therefore, the invention contemplates fusion polypeptides comprising the minimal portion of the extracellular domain necessary to
- 15 bind the cytokine as the cytokine binding portion.

The invention comprises a "specificity determining component" of a cytokine's receptor and a "signal transducing component" of the cytokine's receptor. Regardless of the nomenclature used to designate a particular

- 20 component or subunit of a cytokine receptor, one skilled in the art would recognize which component or subunit of a receptor is responsible for determining the cellular target of the cytokine, and thus would know which component constitutes the "specificity determining component."

- 25 Similarly, regardless of the nomenclature used, one of skill in the art would know which component or subunit of a receptor would constitute the "signal transducing component." As used herein, the "signal transducing component" is a component of the native receptor which is not the specificity determining component and which, does not bind or weakly binds the
- 30 cytokine in the absence of the specificity determining component. In the

native receptor, the “signal transducing component” may participate in signalling.

For example, while some cytokine receptors have components designated α

5 and β , the IL-4 receptor has a signal transducing component referred to as IL-
2R γ . However, regardless of what name is associated with that component,
one skilled in the art would know which component of the IL-4 receptor is
the signal transducing component. Thus to practice the present invention
and create a high affinity trap for IL-4, one of skill in the art would create an
10 isolated nucleic acid comprising a nucleotide sequence encoding a first fusion
polypeptide component comprising the amino acid sequence of the cytokine
binding portion of the extracellular domain of the specificity determining
component of the IL-4 receptor (IL-4R α); a nucleotide sequence encoding a
second fusion polypeptide component comprising the amino acid sequence of
15 the cytokine binding portion of the extracellular domain of the signal
transducing component of the IL-4 receptor (IL-2R γ); and a nucleotide
sequence encoding a third fusion polypeptide component comprising the
amino acid sequence of a multimerizing component (for example, an Fc
domain of IgG) to create a high affinity trap for IL-4.

20

Some further examples of the receptor components that may be used to
prepare cytokine antagonists according to the invention are set forth in Table
1. The Table 1 sets forth, by way of example but not by way of limitation,
some of the varied nomenclature used in the scientific literature to describe
25 those components which function as specificity determining components and
those which function as signal transducing components of certain cytokine
receptors.

TABLE 1

<u>Cytokine</u>	<u>Specificity determining Component</u>	<u>Signal transducing Component</u>
Interleukin-1 (IL-1)	Type I IL-1R (ref. 8) Type II IL-1R (ref. 8) IL-1RI (ref. 11) IL-1RII (ref. 11)	IL-1R AcP (refs. 8, 11)
Interleukin-2 (IL-2)	α -subunit (ref. 2) α -chain (ref. 3) IL-2R α (ref. 1)	β -chain (ref. 3) β -subunit (ref. 2) γ -chain (ref. 3) IL-2R β (refs. 1, 10) IL-2R γ (refs. 1, 10)
Interleukin-3 (IL-3)	IL-3R α (ref. 1)	β_c (ref. 1)
Interleukin-4 (IL-4)	IL-4R (ref. 1)	γ -chain (ref. 3) IL-2R γ (ref. 1)
Interleukin-5 (IL-5)	IL-5R α (ref. 1)	β_c (ref. 1) β -subunit (ref. 2) β -chain (ref. 3) β -receptor component (ref. 5)

TABLE 1 (CONT'D)

<u>Cytokine</u>	<u>Specificity determining Component</u>	<u>Signal transducing Component</u>
Granulocyte macrophage-colony stimulating factor (GM-CSF)	α -receptor component (ref. 5) α -subunit (ref. 2) GMR α (refs. 1, 2) β_c (ref. 1) GMR β (refs. 1, 2)	β -receptor component (ref. 5) β -subunit (ref. 2) β -chain (ref. 3) β_c (ref. 1) GMR β (refs. 1, 2)
Leukemia inhibitory factor (LIF)	LIFBP (ref. 1) α -receptor component (ref. 5)	gp130 (refs. 1, 3) β - receptor component (ref. 5)
Interleukin-11 (IL-11)	α -chain (ref. 4) NR1 (ref. 4)	gp130 (ref. 4)
Interleukin-15 (IL-15)	IL-15R α (ref. 10)	IL-2R β (ref. 10) IL-2R γ (ref. 10)
Interferon- γ (IFN γ)	IFN- γ R (ref. 7) IFN- γ R1 (ref. 7)	AF-1 (ref. 7) IFN- γ R2 (ref. 7)
TGF β	Type II (refs. 6, 9)	Type I (refs. 6, 9)

Only a few of the multitude of references are cited in Table 1, and they are set forth as follows:

1. Sato and Miyajima, Current Opinions in Cell Biology 6: 174-179 (1994) -
5 See page 176, lines 9-16;
2. Miyajima, et al., Annual Review of Immunology 10: 295-331 (1992) -
See page 295, line 4 to page 296, line 1; page 305, last paragraph;
3. Kondo, et al, Science 262: 1874-1877 (1993) - See page 1874, cols. 1 & 2;
4. Hilton, et al, EMBO Journal 13: 4765-4775 (1994) - See page 4766, col. 1,
10 lines 20 24;
5. Stahl and Yancopoulos, Cell 74: 587-590 (1993) - See page 587, column 2,
lines 15-22;
6. Bassing, et al, Journal of Biological Chemistry 269: 14861-14864 (1994) -
See page 14861, col. 2, lines 1-9 and 21-28;
- 15 7. Kotenko, et al, Journal of Biological Science 270: 20915-20921 (1995) - See
page 20915, lines 1-5 of the abstract;
8. Greenfeder, et al., Journal of Biological Chemistry 270: 13757-13765
(1995) - See page 13757, col. 1, line 6 to col. 2, line 3 and col. 2, lines 10-12; page
13764, col. 2, last 3 lines and page 13765, col. 1, lines 1-7;
- 20 9. Lebrun and Vale, Molecular Cell Biology 17: 1682-1691 (1997) - See page
1682, Abstract lines 2-6;
10. Kennedy and Park, Journal of Clinical Immunology 16: 134-143 (1996) -
See page 134, lines 1-7 of the abstract; page 136, col 2., lines 1-5;
11. Wesche, et al., Journal of Biological Chemistry 272: 7727-7731 (1997) See
25 page 7731, lines 20-26.

Kotenko, et al. recently identified the IL-10R2 (IL-10R β) chain which is reported to serve as an accessory chain that is essential for the active IL-10 receptor complex and for initiating IL-10 induced signal transduction events
30 (S.V. Kotenko, et al., The EMBO Journal, 1997, Vol. 16: 5894-5903). Additional

cytokines and their receptors are described in Appendix II, page A:9 of Immunobiology, The Immune System In Health and Disease, 2nd Edition, by Charles A. Janeway, Jr. and Paul Travers, published by Current Biology Ltd./Garland Publishing Inc., copyright 1996.

5

In preparing the nucleic acid sequence encoding the fusion polypeptide of the invention, the first, second, and third components of the fusion polypeptide are encoded in a single strand of nucleotides which, when expressed by a host vector system, produces a monomeric species of the fusion polypeptide. The 10 monomers thus expressed then multimerize due to the interactions between the multimerizing components (the third fusion polypeptide components). Producing the fusion polypeptides in this manner avoids the need for purification of heterodimeric mixtures that would result if the first and second components were produced as separate molecules and then 15 multimerized. For example, U.S. Patent No. 5,470,952 issued November 28, 1995 describes the production of heterodimeric proteins that function as CNTF or IL-6 antagonists. The heterodimers are purified from cell lines cotransfected with the appropriate alpha and beta components. Heterodimers are then separated from homodimers using methods such as passive elution 20 from preparative, nondenaturing polyacrylamide gels or by using high pressure cation exchange chromatography. The need for this purification step is avoided by the methods of the present invention.

In addition, PCT International Application WO 96/11213 published 18 April 25 1996 entitled Dimeric IL-4 Inhibitors states that the applicant has prepared homodimers in which two IL-4 receptors are bound by a polymeric spacer and has prepared heterodimers in which an IL-4 receptor is linked by a polymeric spacer to an IL-2 receptor gamma chain. The polymeric spacer described is polyethylene glycol (PEG). The two receptor components, IL-4R and IL- 30 2Rgamma are separately expressed and purified. Pegylated homodimers and heterodimers are then produced by joining the components together using bi-

functional PEG reagents. It is an advantage of the present invention that it avoids the need for such time consuming and costly purification and pegylation steps.

- 5 In one embodiment of the invention, the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component. In another embodiment of the invention, the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component. Further embodiments of the
- 10 invention may be prepared in which the order of the first, second and third fusion polypeptide components is rearranged. For example, if the nucleotide sequence encoding the first component is designated 1, the nucleotide sequence encoding the second component is designated 2, and the nucleotide sequence of the third component is designated 3, then the order of the
- 15 components in the isolated nucleic acid of the invention as read from 5' to 3' may be any of the following six combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

In further embodiments of the invention, the cytokine bound by the fusion

- 20 polypeptide may be a member of the hematopoietin family of cytokines selected from the group consisting of interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9, interleukin-11, interleukin-13, interleukin-15, granulocyte macrophage colony stimulating factor, oncostatin M, and leukemia inhibitory factor.

25

In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the interferon family of cytokines selected from the group consisting of IFN-gamma, IFN-alpha, and IFN-beta.

In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the immunoglobulin superfamily of cytokines selected from the group consisting of B7.1 (CD80) and B7.2 (B70).

- 5 In still further embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the TNF family of cytokines selected from the group consisting of TNF-alpha, TNF-beta, LT-beta, CD40 ligand, Fas ligand, CD 27 ligand, CD 30 ligand, and 4-1BBL.
- 10 In additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a cytokine selected from the group consisting of interleukin-1, interleukin-10, interleukin-12, interleukin-14, interleukin-18, and MIF.
- 15 Because specificity determination and signal transduction occurs by a similar mechanism in the TGF- β /BMP family of cytokines (See D. Kingsley, Genes & Development, 1994, 8: 133-146; J. Wrana, Miner Electrolyte Metab, 24: 120-130 (1998); R. Derynck and X. Feng, Biochimica et Biophysica Acta 1333 (1997) F105-F150; and J. Massague and F. Weis-Garcia, "Serine/threonine Kinase
- 20 Receptors: Mediators of Transforming Growth Factor Beta Family Signals" In Cancer Surveys, Vol. 27: Cell Signalling, 1996, Imperial Cancer Research Fund) the present invention may be used to produce high affinity antagonists for cytokines that are members of the TGF- β /BMP family.
- 25 Therefore, in additional embodiments of the invention, the cytokine bound by the fusion polypeptide may be a member of the TGF- β /BMP family selected from the group consisting of TGF- β 1, TGF- β 2, TGF- β 3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-15, BMP-16, endometrial bleeding associated factor (EBAF), growth
- 30 differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8,

GDF-9, GDF-12, GDF-14, mullerian inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.

In alternative embodiments of the invention, the specificity determining component, the signal transducing component, or both, may be substituted for by a single chain Fv. A single chain Fv (scFv) is a truncated Fab having only the V region of a heavy chain linked by a stretch of synthetic peptide to a V region of a light chain. See, for example, US Patent Nos. 5,565,332; 5,733,743; 5,837,242; 5,858,657; and 5,871,907 assigned to Cambridge Antibody Technology Limited incorporated by reference herein. Thus the present invention contemplates, for example, an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor; a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of an scFv capable of binding the cytokine at a site different from the site at which the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor binds; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component. Alternatively, the specificity determining component may be substituted for by a scFv that binds to a site on the cytokine different from the site at which the signal transducing component binds. Thus the invention contemplates an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of a scFv that binds to a site on the cytokine different from the site at which the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor binds;

a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

In another embodiment, the invention contemplates an isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of a first scFv that binds to a site on the cytokine; a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of a second scFv that binds to a site on the cytokine different from the site at which the first scFv binds; and a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.

In all of the above described embodiments comprising scFv's, the invention also contemplates embodiments in which the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component; embodiments in which the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component; and further embodiments of the invention in which the order of the first, second and third fusion polypeptide components is rearranged. For example, if the nucleotide sequence encoding the first component is designated 1, the nucleotide sequence encoding the second component is designated 2, and the nucleotide sequence of the third component is designated 3, then the order of the components in the isolated nucleic acid of the invention as read from 5' to 3' may be any of the following six combinations: 1,2,3; 1,3,2; 2,1,3; 2,3,1; 3,1,2; or 3,2,1.

In preferred embodiments of the invention, the multimerizing component comprises an immunoglobulin derived domain. More specifically, the immunoglobulin derived domain may be selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG. In another embodiment, the multimerizing component may be an Fc domain from which the first five amino acids (including a cysteine) have been removed to produce a multimerizing component referred to as Fc(ΔC1). Alternatively, the multimerizing component may be an Fc domain in which a cysteine within the first five amino acids has been substituted for by another amino acid such as, for example, serine or alanine.

The present invention also provides for fusion polypeptides encoded by the isolated nucleic acid molecules of the invention. Preferably, the fusion polypeptides are in multimeric form, due to the function of the third multimerizing component. In a preferred embodiment, the multimer is a dimer. Suitable multimerizing components are sequences encoding an immunoglobulin heavy chain hinge region (Takahashi et al., 1982, *Cell* 29:671-679); immunoglobulin gene sequences, and portions thereof. In a preferred embodiment of the invention, immunoglobulin gene sequences, especially one encoding the Fc domain, are used to encode the third multimerizing component.

The present invention also contemplates a vector which comprises the nucleic acid molecule of the invention as described herein.

Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the

expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion polypeptide. The suitable host cell may be a bacterial cell such as E. coli, a yeast cell, such as Pichia pastoris, an insect cell, such as Spodoptera frugiperda, or a mammalian cell, such as a COS, CHO,

5 293, BHK or NS0 cell.

The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector systems described herein, under conditions permitting production of the fusion

10 polypeptide and recovering the fusion polypeptide so produced.

The present invention provides novel antagonists which are based on receptor components that are shared by cytokines such as the CNTF family of cytokines.

15 The invention described herein contemplates the production of antagonists to any cytokine that utilizes an α specificity determining component which, when combined with the cytokine, binds to a first β signal transducing component to form a nonfunctional intermediate which then binds to a

20 second β signal transducing component causing β - receptor dimerization and consequent signal transduction. According to the invention, the soluble α specificity determining component of the receptor (sR α) and the extracellular domain of the first β signal transducing component of the cytokine receptor (β 1) are combined to form heterodimers (sR α : β 1) that act as antagonists to the

25 cytokine by binding the cytokine to form a nonfunctional complex.

As described in Example 1, CNTF and IL-6 share the β 1 receptor component gp130. The fact that CNTF forms an intermediate with CNTFR α and gp130

can be demonstrated (Example 1) in cells lacking LIFR β , where the complex of CNTF and CNTFR α binds gp130, and prevents homodimerization of gp130 by IL-6 and IL-6R α , thereby blocking signal transduction. These studies provide the basis for the development of the IL-6 antagonists described
5 herein, as they show that if, in the presence of a ligand, a nonfunctional intermediate complex, consisting of the ligand, its α receptor component and its β 1 receptor component, can be formed, it will effectively block the action of the ligand. Other cytokines may use other β 1 receptor components, such as LIFR β , which may also be used to produce antagonists according to the
10 present invention.

Thus for example, in one embodiment of the invention, effective antagonists of IL-6 or CNTF consist of heterodimers of the extracellular domains of the α specificity determining components of their receptors (sIL-6R α and sCNTFR α
15 respectively) and the extracellular domain of gp130. The resultant heterodimers, which are referred to hereinafter as sIL-6R α : β 1 and sCNTFR α : β 1 respectively, function as high-affinity traps for IL-6 or CNTF, respectively, thus rendering the cytokine inaccessible to form a signal transducing complex with the native membrane-bound forms of their
20 receptors.

Although soluble ligand binding domains from the extracellular portion of receptors have proven to be somewhat effective as traps for their ligands and thus act as antagonists [Bargetzi, et al., Cancer Res. 53:4010-4013 (1993); , et al.,
25 Proc. Natl. Acad. Sci. USA 89: 8616-8620 (1992); Mohler, et al., J. Immunol. 151: 1548-1561 (1993); Narazaki, et al., Blood 82: 1120-1126 (1993)], the IL-6 and CNTF receptors are unusual in that the α receptor components constitute

ligand binding domains that, in concert with their ligands, function effectively in soluble form as receptor agonists [Davis, et al. Science 259:1736-1739 (1993); Taga, et al., Cell 58: 573-581 (1989)]. The sR α : β 1 heterodimers prepared according to the present invention provide effective traps for their 5 ligands, binding these ligands with affinities in the picomolar range (based on binding studies for CNTF to PC12D cells) without creating functional intermediates. The technology described herein may be applied to develop a cytokine trap for any cytokine that utilizes an α -component that confers specificity, as well as a β component which, when bound to the α -specificity 10 component, has a higher affinity for the cytokine than either component alone. Accordingly, antagonists according to the invention include antagonists of interleukins 1 through 5 [IL-1, Greenfeder, et al. J. Biol. Chem. 270:13757-13765 (1995); Guo, et al. J. Biol. Chem. 270:27562-27568 (1995)], IL-2; [Taniguchi, et al. European Patent Nos. 0386289-A and 0386304-A (1990); 15 Takeshita, et al. Science 257:379-382 (1992)]; IL-3; [Kitamura, et al. Cell 66:1165-1174 (1991)], IL-4; [Idzerda, et al. J. Exp. Med. 171:861-873 (1990)], IL-5; [Taverneir, et al. Cell 66:1175-1184 (1991)], IL-11 [(Cherel, et al. Direct 20 Submission to EMBL/GenBank/DDBJ databases; accession No. Z38102)], interleukin 15 [IL-15; Hemar, et al. J. Cell Biol. 1295:55-64 (1995); Taniguchi, et 25 al. European Patent Nos. 0386289-A and 0386304-A (1990); Takeshita, et al. Science 257:379-382 (1992)], granulocyte-macrophage colony stimulating factor [GM-CSF; Hayashida, et al. Proc. Natl. Acad. Sci. U.S.A. 97:9655-9659 (1990)], LIF, gamma interferon [IFN γ ; Aguet, et al. Cell 55:273-280 (1988); Soh, et al. Cell 76:793-802 (1994)], and transforming growth factor beta [TGF β ; Inagaki, et al. Proc. Natl. Acad. Sci. USA 90:5359-5363 (1993)].

The α and β receptor extracellular domains may be prepared using methods

known to those skilled in the art. The CNTFR α receptor has been cloned, sequenced and expressed [Davis, et al. (1991) Science 253:59-63 which is incorporated by reference in its entirety herein]. The cloning of LIFR β and gp130 are described in Gearing et al. in EMBO J. 10:2839-2848 (1991), Hibi, et al. 5 Cell 63:1149-1157 (1990) and in published PCT application WO 93/10151 published May 27, 1993, all of which are incorporated by reference in their entirety herein.

The receptor molecules useful for practicing the present invention may be prepared by cloning and expression in a prokaryotic or eukaryotic 10 expression system. The recombinant receptor gene may be expressed and purified utilizing any number of methods. The gene encoding the factor may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pCP110.

The recombinant factors may be purified by any technique which allows 15 for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, conventional ion exchange 20 chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration may be used.

The sR α : β heterodimeric receptors may be engineered using known fusion regions, as described in published PCT application WO 93/10151 published May 27, 1993 entitled "Receptor for Oncostatin M and Leukemia 25 Inhibitory Factor" which describes production of β receptor heterodimers, or they may be prepared by crosslinking of extracellular domains by chemical means. The domains utilized may consist of the entire extracellular domain

of the α and β components, or they may consist of mutants or fragments thereof that maintain the ability to form a complex with its ligand and other components in the sR α : β 1 complex. For example, as described below in example 4, IL-6 antagonists have been prepared using gp130 that is lacking its
5 three fibronectin-like domains.

In one embodiment of the invention, the extracellular domains are engineered using leucine zippers. The leucine zipper domains of the human transcription factors c-jun and c-fos have been shown to form stable heterodimers [Busch and Sassone-Corsi, Trends Genetics 6: 36-40 (1990);
10 Gentz, et al., Science 243: 1695-1699 (1989)] with a 1:1 stoichiometry. Although jun-jun homodimers have also been shown to form, they are about 1000-fold less stable than jun-fos heterodimers. Fos-fos homodimers have not been detected.

The leucine zipper domain of either c-jun or c-fos are fused in frame at
15 the C-terminus of the soluble or extracellular domains of the above mentioned receptor components by genetically engineering chimeric genes. The fusions may be direct or they may employ a flexible linker domain, such as the hinge region of human IgG, or polypeptide linkers consisting of small amino acids such as glycine, serine, threonine or alanine, at various lengths
20 and combinations. Additionally, the chimeric proteins may be tagged by His-His-His-His-His (His6),[SEQ. ID NO. 1] to allow rapid purification by metal-chelate chromatography, and/or by epitopes to which antibodies are available, to allow for detection on western blots, immunoprecipitation, or activity depletion/blocking in bioassays.

25 In another embodiment, as described below in Example 3. the sR α : β 1 heterodimer is prepared using a similar method, but using the Fc-domain of human IgG1 [Aruffo, et al., Cell 67:35-44 (1991)]. In contrast to the latter,

formation of heterodimers must be biochemically achieved, as chimeric molecules carrying the Fc-domain will be expressed as disulfide-linked homodimers. Thus, homodimers may be reduced under conditions that favor the disruption of inter-chain disulfides but do not effect intra-chain disulfides. Then monomers with different extracellular portions are mixed in equimolar amounts and oxidized to form a mixture of homo- and heterodimers. The components of this mixture are separated by chromatographic techniques. Alternatively, the formation of this type of heterodimers may be biased by genetically engineering and expressing molecules that consist of the soluble or extracellular portion of the receptor components followed by the Fc-domain of hIgG, followed by either the c-jun or the c-fos leucine zippers described above [Kostelny, et al., J. Immunol. 148: 1547-1553 (1992)]. Since these leucine zippers form predominately heterodimers, they may be used to drive formation of the heterodimers where desired. As for the chimeric proteins described using leucine zippers, these may also be tagged with metal chelates or an epitope. This tagged domain can be used for rapid purification by metal-chelate chromatography, and/or by antibodies, to allow for detection on western blots, immunoprecipitation, or activity depletion/blocking in bioassays.

In additional embodiments, heterodimers may be prepared using other immunoglobulin derived domains that drive the formation of dimers. Such domains include, for example, the heavy chains of IgG (Cy1 and Cy4), as well as the constant regions of kappa (κ) and lambda (λ) light chains of human immunoglobulins. The heterodimerization of Cy with the light chain occurs between the CH1 domain of Cy and the constant region of the light chain (CL), and is stabilized by covalent linking of the two domains via a single disulfide bridge. Accordingly, as described in Example 4, constructs may be

prepared using these immunoglobulin domains. Alternatively, the immunoglobulin domains include domains that may be derived from T cell receptor components which drive dimerization,

In another embodiment of the invention the sR α : β 1 heterodimers are
5 prepared by expression as chimeric molecules utilizing flexible linker loops. A DNA construct encoding the chimeric protein is designed such that it expresses two soluble or extracellular domains fused together in tandem ("head to head") by a flexible loop. This loop may be entirely artificial (e.g. polyglycine repeats interrupted by serine or threonine at a certain interval) or
10 "borrowed" from naturally occurring proteins (e.g. the hinge region of hIgG). Molecules may be engineered in which the order of the soluble or extracellular domains fused is switched (e.g. sIL6R α /loop/sgp130 or sgp130/loop/sIL-6R α) and/or in which the length and composition of the loop is varied, to allow for selection of molecules with desired characteristics.

15 Alternatively, the heterodimers made according to the present invention may be purified from cell lines cotransfected with the appropriate α and β components. Heterodimers may be separated from homodimers using methods available to those skilled in the art. For example, limited quantities of heterodimers may be recovered by passive elution from
20 preparative, nondenaturing polyacrylamide gels. Alternatively, heterodimers may be purified using high pressure cation exchange chromatography. Excellent purification has been obtained using a Mono S cation exchange column.

25 In addition to sR α : β 1 heterodimers that act as antagonists by binding free CNTF or IL-6, the present invention also contemplates the use of engineered, mutated versions of IL-6 with novel properties that allow it to bind to IL-6R α and a single gp130 molecule, but fail to engage the second

gp130 to complete β component homodimerization, and thus act as an effective IL-6 antagonist on any IL-6 responsive cell. Our model for the structure of the IL-6 and CNTF receptor complexes indicates that these cytokines have distinct sites for binding the α , β_1 , and β_2 receptor components [Stahl and Yancopoulos, Cell 74: 587-590 (1993)]. Mutations of critical amino acid residues comprising each of these sites gives rise to novel molecules which have the desired antagonistic properties. Ablation of the β_1 site would give a molecule which could still bind to the α receptor component but not the β_1 component, and thereby comprise an antagonist with nanomolar affinity. Mutations of critical amino acid residues comprising the β_2 site of IL-6 (IL-6 β_2^-) would give a molecule that would bind to IL-6R α and the first gp130 monomer, but fail to engage the second gp130 and thus be functionally inactive. Similarly, mutations of the CNTF β_2 site would give a molecule (CNTF β_2^-) that would bind CNTFR α and gp130, but fail to engage LIFR β , thereby antagonizing CNTF action by forming the non-functional β_1 intermediate. Based on the binding results described above where CNTF forms the β_1 intermediate with high affinity, both CNTF β_2^- and IL-6 β_2^- would constitute antagonists with affinity in the range of 10 pM.

A variety of means are used to generate and identify mutations of IL-6 or CNTF that have the desired properties. Random mutagenesis by standard methods of the DNA encoding IL-6 or CNTF may be used, followed by analysis of the collection of products to identify mutated cytokines having the desired novel properties as outlined below. Mutagenesis by genetic engineering has been used extensively in order to elucidate the structural organization of functional domains of recombinant proteins. Several different approaches have been described in the literature for carrying out deletion or substitution mutagenesis. The most successful appear to be

alanine scanning mutagenesis [Cunningham and Wells (1989), Science 244: 1081-1085] and homolog-scanning mutagenesis [Cunningham, et al., (1989), Science 243:1330-1336].

Targeted mutagenesis of the IL-6 or CNTF nucleic acid sequences using
5 such methods can be used to generate CNTF β 2- or IL-6 β 2- candidates. The choice of regions appropriate for targeted mutagenesis is done systematically, or determined from studies whereby panels of monoclonal antibodies against each factor are used to map regions of the cytokine that might be exposed after binding of the cytokine to the α receptor component alone, or to the $\alpha\beta$ 1
10 heterodimeric soluble receptors described above. Similarly, chemical modification or limited proteolysis of the cytokine alone or in a complex bound to the α receptor component or the $\alpha\beta$ 1 heterodimeric soluble receptors described above, followed by analysis of the protected and exposed regions could reveal potential β 2 binding sites.

15 Assays for identifying CNTF or IL-6 mutants with the desired properties involve the ability to block with high affinity the action of IL-6 or CNTF on appropriately responsive cell lines [Davis, et al., Science 259: 1736-1739 (1993); Murakami, et al., Proc. Natl. Acad. Sci. USA 88: 11349-11353 (1991)]. Such assays include cell proliferation, survival, or DNA synthesis
20 driven by CNTF or IL-6, or the construction of cell lines where binding of factor induces production of reporters such as CAT or β -galactosidase [Savino, et al., Proc. Natl. Acad. Sci. USA 90: 4067-4071 (1993)].

25 Alternatively, the properties of various mutants may be assessed with a receptor-based assay. One such assay consists of screening mutants for their ability to bind the sR α : β 1 receptor heterodimers described above using epitope-tagged [Davis et al., Science 253: 59-63 (1991)] sR α : β 1 reagents. Furthermore, one can probe for the presence or absence of the β 2 site by

assessing whether an epitope-tagged soluble β 2 reagent will bind to the cytokine in the presence of the β 1 heterodimer. For example, CNTF only binds to LIFR β (the β 2 component) in the presence of both CNTFR α and gp130 [Davis, et al. Science 260: 1805-1808 (1993); Stahl, et al. J. Biol. Chem. 268: 5 7628-7631 (1993)]. Thus a soluble LIFR β reagent would only bind to CNTF in the presence of the soluble sR α : β 1 dimer sCNTFR α : β 1. For IL-6, the sR α : β 1 reagent would be IL-6R α : β 1, and the probe for the β 2 site would be epitope-tagged sgp130. Thus β 2- mutants of CNTF would be identified as those that bound the sR α : β 1 reagent, demonstrating that the α and β 1 site of the 10 cytokine were intact, yet failed to bind the β 2 reagent.

In addition, the present invention provides for methods of detecting or measuring the activity of potential β 2- mutants by measuring the phosphorylation of a β -receptor component or a signal transduction 15 component selected from the group consisting of Jak1, Jak2 and Tyk2 or any other signal transduction component, such as the CLIPs, that are determined to be phosphorylated in response to a member of the CNTF family of cytokines.

A cell that expresses the signal transduction component(s) described 20 herein may either do so naturally or be genetically engineered to do so. For example, Jak1 and Tyk-2-encoding nucleic acid sequences obtained as described in Velazquez, et al., Cell, Vol. 70:313-322 (1992), may be introduced into a cell by transduction, transfection, microinjection, electroporation, via a transgenic animal, etc., using any known method known in the art.

25 According to the invention, cells are exposed to a potential antagonist and the tyrosine phosphorylation of either the β -component(s) or the signal transduction component(s) are compared to the tyrosine phosphorylation of

the same component(s) in the absence of the potential antagonist.

In another embodiment of the invention, the tyrosine phosphorylation that results from contacting the above cells with the potential antagonist is compared to the tyrosine phosphorylation of the same cells exposed to the parental CNTF family member. In such assays, the cell must either express the extracellular receptor (α -component) or the cells may be exposed to the test agent in the presence of the soluble receptor component.

Thus, for example, in an assay system designed to identify agonists or antagonists of CNTF, the cell may express the α - component CNTFR α , the β -components gp130 and LIFR β and a signal transducing component such as Jak1. The cell is exposed to test agents, and the tyrosine phosphorylation of either the β - components or the signal transducing component is compared to the phosphorylation pattern produced in the presence of CNTF.

Alternatively, the tyrosine phosphorylation which results from exposure to a test agent is compared to the phosphorylation which occurs in the absence of the test agent. Alternatively, an assay system, for example, for IL-6 may involve exposing a cell that expresses the β -component gp130 and a signal transducing protein such as Jak1, Jak2 or Tyk2 to a test agent in conjunction with the soluble IL-6 receptor.

In another embodiment of the invention the above approaches are used to develop a method for screening for small molecule antagonists that act at various steps in the process of ligand binding, receptor complex formation, and subsequent signal transduction. Molecules that potentially interfere with ligand-receptor interactions are screened by assessing

interference of complex formation between the soluble receptors and ligand as described above. Alternatively, cell-based assays in which IL-6 or CNTF induce response of a reporter gene are screened against libraries of small

molecules or natural products to identify potential antagonists. Those molecules showing antagonist activity are rescreened on cell-based assays responding to other factors (such as GM-CSF or factors like Neurotrophin-3 that activate receptor tyrosine kinases) to evaluate their specificity against the

- 5 CNTF/IL-6/OSM/LIF family of factors. Such cell-based screens are used to identify antagonists that inhibit any of numerous targets in the signal transduction process.

In one such assay system, the specific target for antagonists is the interaction of the Jak/Tyk family of kinases [Firnbach-Kraft, Oncogene 5: 10 1329-1336 (1990); Wilks, et al., Mol. Cell. Biol. 11:2057-2065 (1991)] with the receptor β subunits. As described above, LIFR β and gp130 preassociate with members of the Jak/Tyk family of cytoplasmic protein tyrosine kinases, which become activated in response to ligand-induced β component dimerization Stahl, et al. Science 263:92-95 (1993). Thus small molecules that could enter 15 the cell cytoplasm and disrupt the interaction between the β component and the Jak/Tyk kinase could potentially block all subsequent intracellular signaling. Such activity could be screened with an *in vitro* scheme that assessed the ability of small molecules to block the interaction between the relevant binding domains of purified β component and Jak/Tyk kinase.

- 20 Alternatively, one could easily screen for molecules that could inhibit a yeast-based assay of β component binding to Jak/Tyk kinases using the two-hybrid interaction system [Chien, et al., Proc. Natl. Acad. Sci. 88: 9578-9582 (1991)]. In such a system, the interaction between two proteins (β component and Jak/Tyk kinase or relevant domains thereof in this example) induces 25 production of a convenient marker such as β - galactosidase. Collections of small molecules are tested for their ability to disrupt the desired interaction without inhibiting the interaction between two control proteins. The

advantage of this screen would be the requirement that the test compounds enter the cell before inhibiting the interaction between the β component and the Jak/Tyk kinase.

The CNTF family antagonists described herein either bind to, or
5 compete with the cytokines CNTF and IL-6. Accordingly, they are useful for treating diseases or disorders mediated by CNTF or IL-6. For example, therapeutic uses of IL-6 antagonists would include the following:

- 1) In osteoporosis, which can be exacerbated by lowering of estrogen levels in post-menopausal women or through ovariectomy, IL-6 appears to be
10 a critical mediator of osteoclastogenesis, leading to bone resorption [Horowitz, Science 260: 626-627 (1993); Jilka, et al., Science 257: 88-91 (1992)]. Importantly, IL-6 only appears to play a major role in the estrogen-depleted state, and apparently is minimally involved in normal bone maintenance. Consistent with this, experimental evidence indicates that function-blocking antibodies
15 to IL-6 can reduce the number of osteoclasts [Jilka, et al. Science 257: 88-91 (1992)]. While estrogen replacement therapy is also used, there appear to be side effects that may include increased risk of endometrial and breast cancer. Thus, IL-6 antagonists as described herein would be more specific to reduce osteoclastogenesis to normal levels.
- 20 2) IL-6 appears to be directly involved in multiple myeloma by acting in either an autocrine or paracrine fashion to promote tumor formation [van Oers, et al., Ann Hematol. 66: 219-223 (1993)]. Furthermore, the elevated IL-6 levels create undesirable secondary effects such as bone resorption, hypercalcemia, and cachexia; in limited studies function-blocking antibodies
25 to IL-6 or IL-6Ra have some efficacy [Klein, et al., Blood 78: 1198-1204 (1991); Suzuki, et al., Eur. J. Immunol. 22:1989-1993 (1992)]. Therefore, IL-6 antagonists as described herein would be beneficial for both the secondary

effects as well as for inhibiting tumor growth.

3) IL-6 may be a mediator of tumor necrosis factor (TNF) that leads to cachexia associated with AIDS and cancer [Strassmann, et al., J. Clin. Invest. 89: 1681-1684 (1992)], perhaps by reducing lipoprotein lipase activity in adipose tissue [Greenberg, et al., Cancer Research 52: 4113-4116 (1992)]. Accordingly, antagonists described herein would be useful in alleviating or reducing cachexia in such patients.

Effective doses useful for treating these or other CNTF family related diseases or disorders may be determined using methods known to one skilled in the art [see, for example, Fingl, et al., *The Pharmacological Basis of Therapeutics*, Goodman and Gilman, eds. Macmillan Publishing Co., New York, pp. 1-46 ((1975))]. Pharmaceutical compositions for use according to the invention include the antagonists described above in a pharmacologically acceptable liquid, solid or semi-solid carrier, linked to a carrier or targeting molecule (e.g., antibody, hormone, growth factor, etc.) and/or incorporated into liposomes, microcapsules, and controlled release preparation (including antagonist expressing cells) prior to administration *in vivo*. For example, the pharmaceutical composition may comprise one or more of the antagonists in an aqueous solution, such as sterile water, saline, phosphate buffer or dextrose solution. Alternatively, the active agents may be comprised in a solid (e.g. wax) or semi-solid (e.g. gelatinous) formulation that may be implanted into a patient in need of such treatment. The administration route may be any mode of administration known in the art, including but not limited to intravenously, intrathecally, subcutaneously, by injection into involved tissue, intraarterially, intranasally, orally, or via an implanted device.

Administration may result in the distribution of the active agent of the

invention throughout the body or in a localized area. For example, in some conditions which involve distant regions of the nervous system, intravenous or intrathecal administration of agent may be desirable. In some situations, an implant containing active agent may be placed in or near the lesioned area.

- 5 Suitable implants include, but are not limited to, gelfoam, wax, or microparticle-based implants.

EXAMPLE 1: CNTF COMPETES WITH IL-6 FOR BINDING TO GP130

MATERIALS AND METHODS

10 Materials. A clone of PC12 cells that respond to IL-6 (PC12D) was obtained from DNAX. Rat CNTF was prepared as described [Masiakowski, et al., J. Neurochem. 57:1003-10012 (1991)]. IL-6 and sIL-6R α were purchased from R & D Systems. Antisera was raised in rabbits against a peptide derived from a region near the C-terminus of gp130 (sequence:

15 CGTEGQVERFETVGME) [SEQ. ID. NO. 2] by the method described (Stahl, et al. J. Biol. Chem. 268:7628-7631 (1993). Anti-phosphotyrosine monoclonal 4G10 was purchased from UBI, and reagents for ECL from Amersham.

20 Signal Transduction Assays. Plates (10 cm) of PC12D were starved in serum-free medium (RPMI 1640 + glutamine) for 1 hour, then incubated with IL-6 (50 ng/mL) + sIL-6R (1 mg/mL) in the presence or absence of added rat CNTF at the indicated concentrations for 5 minutes at 37°C. Samples were then subjected to anti-gp130 immunoprecipitation, SDS PAGE, and anti-phosphotyrosine immunoblotting as described (Stahl, et al. J. Biol. Chem. 268:7628-7631 (1993)).

25 RESULTS

The ability of CNTF to block IL-6 responses was measured using a PC12 cell line (called PC12D) that expresses IL-6Ra, gp130, and CNTFR α , but not

LIFR β . As one would predict, these cells respond to IL-6, but not to CNTF (Fig. 2) since LIFR β is a required component for CNTF signal transduction [Davis, et al., Science 260: 59-63 (1993)]. In accordance with results on other cell lines [Ip, et al., Cell 69: 1121-1132 (1992)], PC12D cells give tyrosine

5 phosphorylation of gp130 (as well as a variety of other proteins called CLIPs) in response to 2 nM IL-6 (Fig. 2). Addition of recombinant soluble IL-6R α (sIL-6R α) enhances the level of gp130 tyrosine phosphorylation, as has been reported in some other systems [(Taga, et al., Cell 58: 573-581 (1989)]. However, addition of 2 nM CNTF simultaneously with IL-6 severely 10 diminishes the tyrosine phosphorylation of gp130. Although a slight gp130 phosphorylation response remains in the presence of CNTF, IL-6, and sIL-6R α , it is eliminated if the CNTF concentration is increased fourfold to 8 nM. Thus, in IL-6 responsive cells that contain CNTFR α but no LIFR β , CNTF is a rather potent antagonist of IL-6 action.

15

EXAMPLE 2. BINDING OF CNTF TO THE CNTFR α : β

MATERIALS AND METHODS

20 Scatchard Analysis of CNTF Binding. ^{125}I -CNTF was prepared and purified as described [Stahl et al. JBC 268: 7628-7631 (1993)]. Saturation binding studies were carried out in PC12 cells, using concentrations of ^{125}I -CNTF ranging from 20 pM to 10 nM. Binding was performed directly on a monolayer of cells. Medium was removed from wells and cells were washed once with 25 assay buffer consisting of phosphate buffered saline (PBS; pH 7.4), 0.1 mM bacitracin, 1 mM PMSF, 1 mg/ml leupeptin, and 1 mg/ml BSA. Cells were incubated in ^{125}I -CNTF for 2 hours at room temperature, followed by 2 quick

washes with assay buffer. Cells were lysed with PBS containing 1% SDS and counted in a Packard Gamma Counter at 90-95% efficiency. Non-specific binding was defined by the presence of 100-fold excess of unlabelled CNTF. Specific binding ranged from 70% to 95%.

5 RESULTS

The equilibrium constant for binding of CNTF to CNTFR $\alpha:\beta$ 1 was estimated from Scatchard analysis of iodinated CNTF binding on PC12D cells (Figure 3). The data is consistent with a 2 site fit having dissociation constants of 9 pM and 3.4 nM. The low affinity site corresponds to interaction of CNTF with CNTFR α , which has a Kd near 3 nM [(Panayotatos, et al., J. Biol. Chem. 268: 19000-19003 (1993)]. We interpret the high affinity complex as the intermediate containing CNTF, CNTFR α , and gp130. A Ewing sarcoma cell line (EW-1) which does contain CNTFR α , gp130, and LIFR β , and therefore gives robust tyrosine phosphorylation in response to CNTF, displays a very similar two site fit with dissociation constants of 1 nM and 10 pM (Wong, et al., unpublished data). Thus it is apparent that CNTF binds with equally high affinity to a complex containing only CNTFR α and gp130, as it does to a complex which additionally contains LIFR β , thus demonstrating the feasibility of creating the sR $\alpha:\beta$ antagonists described herein.

20

EXAMPLE 3. METHODS OF PRODUCING CYTOKINE LIGAND TRAPS

Virus Stock Production

SF21 insect cells obtained from *Spodoptera frugiperda* were grown at 27C in Gibco SF900 II medium to a density of 1×10^6 cells/mL. The individual virus stock for either GP130-Fc-His6 (Figure 4) or IL6Ra-Fc (Figure 5) was added to the bioreactor to a low multiplicity 0.01-0.1 PFU/cell to begin the

infection. The infection process was allowed to continue for 5-7 days allowing maximum virus replication without incurring substantial cell lysis. The cell suspension was aseptically aliquoted into sterile centrifuge bottles and the cells removed by centrifugation. The cell-free supernatant was collected in
5 sterile bottles and stored at 4C until further use.

The virus titer was determined by plaque assay as described by O'Reilly, Miller and Luckow. The method is carried out in 60mm tissue-culture dishes which are seeded with 2×10^6 cells. Serial dilutions of the virus stock are added to the attached cells and the mixture incubated with rocking to allow
10 the virus to adsorb to individual cells. An agar overlay is added and plates incubated for 5 - 7 days at 27C. Staining of viable cells with neutral red revealed circular plaques resulting which were counted to give the virus titer.

Coinfection of Cells for Protein Production

15 Uninfected SF21 Cells were grown in a 60L ABEC bioreactor containing 40L of SF900 II medium. Temperature was controlled at 27C and the dissolved oxygen level was maintained at 50% of saturation by controlling the flowrate of oxygen in the inlet gas stream. When a density of 2×10^6 cells/mL was reached, the cells were concentrated within the bioreactor to a volume of
20 20L using a low shear steam sterilizable pump and a with tangential flow filtration device with Millipore Prostak 0.65 micron membranes. After concentration fresh sterile growth medium is slowly added to the bioreactor while the filtration system continues to remove the spent growth medium by diafiltration. After two volume exchanges (40L) have been carried out an
25 additional 20L of fresh medium was added to the bioreactor to resuspend the cells to the original volume of 40L. The cell density was determined once again by counting viable cells using a hemacytometer.

The required amount of each virus stock was calculated based on the cell density, virus titer and the desired multiplicity of infection (MOI). Virus stock ratios of 5:1, 5:2, 10:2 and 10:4, IL6Ra-Fc to GP130-Fc-His6 all resulted in production of significant amounts of heterodimer. The ideal virus stock ratio
5 is highly dependent on the ease of purification of the heterodimer from each of the two homodimers. The IL6Ra-Fc homodimer is relatively easy to remove downstream by immobilized metal affinity chromatography. Virus infection ratios have been chosen to minimize the formation of the GP130-Fc-His6 homodimer which is more difficult to clear downstream. The relative
10 amount of GP130-Fc-His6 virus stock chosen for infection has increased with successive batches as the purification method for clearing the resultant homodimer has improved.

The virus stocks were aseptically mixed in a single vessel then transferred to the bioreactor. This results in synchronous infection of the
15 SF21 cells. The infection is allowed to proceed for three to four days, allowing sufficient time for maximal production of the heterodimer protein.

Recovery and Protein A Chromatographic Purification

At the conclusion of the infection phase of the bioreactor process the
20 cells were concentrated in the bioreactor using a 10 ft² Millipore Prostak filter (0.65 micron) pore size. The cell-free permeate passing through the filter was collected in a clean process vessel. At the conclusion of the filtration operation the pH of permeate stream, containing the protein product, was adjusted to 8.0 with 10N NaOH. The resultant precipitate was removed by
25 forcing the extract through a 0.8 micron depth filter (Sartorius), followed by a 0.2 micron filter. Sufficient 0.5M EDTA stock was added to give a final concentration of 5mM. The filtered protein solution was loaded onto a 10 cm

diameter column containing 100-200 mL of Pharmacia Protein A Sepharose 4 Fast Flow, equilibrated with PBS. Protein A has a very high affinity for the Fc-Fc domain of each of the 3 recombinant protein products, allowing them to bind while other proteins in the cell-free extract flow through the column.

5 After loading the column was washed to baseline with PBS containing an additional 350mM NaCl. The IgG-Fc tagged proteins were eluted at low pH, either with 0.5M acetic acid or with a decreasing pH gradient of 0.1M citric acid and 0.2M disodium phosphate buffers. Tris base or disodium phosphate was added to the eluted protein to avoid prolonged exposure to low pH

10 conditions.

The pooled protein was diafiltered into PBS or HEPES buffer for subsequent and derivitized with 1 mM iodoacetamide to protect the exposed sulfhydryl group on the free cysteine near the hinge region of each Fc domain. This prevents disulfide mediated aggregation of proteins. A 6 ft² Millipore spiral wound ultrafiltration membrane with nominal 30 kiloDalton cutoff was used to perform the buffer exchange. The total protein was determined by UV absorbance at 280 nm using the diafiltration buffer as a blank. The relative amounts of heterodimer and two homodimer proteins were determined by SDS PAGE gel electrophoresis using a 6% Tris-Glycine gel (Novex). Gels were Coomasie stained then transferred into destain solution overnight. A Shimadzu scanning densitometer was used to determine the relative intensity of the individual protein bands on the SDS PAGE gel. The peak area ratios are used to compute the fraction of heterodimer and each of the homodimers in the column pool fractions.

25

Immobilized Metal Affinity Chromatographic Purification

The six histidine residues on the C-terminus of the GP130-Fc-His6

fusion protein provides an excellent molecular handle for separation of the heterodimeric IL6 antagonist from the two homodimers. The imidazole group on each of the C-terminal histidines of the GP130-Fc-His₆ moiety has a strong binding constant with several divalent metals, including copper, 5 nickel, zinc, cobalt, iron and calcium. Since the IL6Ra-Fc homodimer has no C-terminal histidine residues, it clearly has the lowest affinity. The IL6Ra-Fc-GP130-Fc-His₆ heterodimer has a single stand set six histidines giving it greater affinity for the metal, while the GP130-Fc-His₆ homodimer has two sets of six histidines each giving it the highest affinity of the three IgG tagged 10 proteins to the metal affinity column. Selective elution of the three proteins with increasing amounts of imidazole in the elution buffer therefore elutes the proteins in the following order:

1. IL6Ra-Fc homodimer
- 15 2. IL6Ra-Fc-GP130-Fc-His heterodimer
3. GP130-Fc-His homodimer

A 26 mm diameter column containing 100 mL of Pharmacia Chelating Sepharose Fast Flow was saturated with a solution of nickel sulfate until a 20 significant green color is observed in the column eluate. The column is then washed with several column volumes of deionized water, then equilibrated with 50 mM HEPES, 40mM imidazole, pH 8.0. The binding of imidazole to the immobilized nickel results in a green to blue color change. Imidazole was added to the protein load to a final concentration of 40mM. Addition of 25 imidazole to the protein load reduces the binding of IL6Ra-Fc homodimer, increasing the surface area available for the remaining two species. After loading, the column was washed with several column volumes of 50 mM

HEPES, 80mM imidazole, pH 8.0 until a steady baseline was reestablished. The heterodimer was selectively eluted with 50 mM HEPES, 150mM imidazole, pH 8.0 over several column volumes. The protein fractions were pooled and diafiltered into PBS as described in the section above.

5

**EXAMPLE 4. ALTERNATIVE METHODS OF CONSTRUCTING
LIGAND TRAPS**

BACKGROUND

10 As described above, receptor activation by CNTF, and analogously by IL-6 and IL-11, follows an ordered sequence of binding events (Figure 6). The cytokine initially binds to its cognate R α with low affinity (K_d = 3 to 10 nM); this is a required step - cells which do not express the cognate R α do not respond to the cognate cytokine. The cytokine•R α complex associates with 15 the first signal transducing component, gp130, to form a high affinity complex (K_d in the order of 10 pM for the CNTF•CNTFR α •gp130 complex). This complex does not transduce signal, as it is the dimerization of the signal transducing components that brings about signaling (Stahl and Yancopoulos, J. Neurobiology 25: 1454-1466 (1994); Stahl et al., Science 267:1349-1353 (1995); 20 Davis et al., Science 260:1805-1808 (1993); Stahl et al., Science 263:92-95 (1994); Murakami, et al. Science 260:1808-1810 (1993). At least in the case of IL-6, the cytokine•R α •signal transducer heterotrimeric complex subsequently associates with another like complex, to form a hexameric complex (Figure 6) (Ward et al., J. Biol. Chem. 269:23286-23289 (1994). The resulting dimerization 25 of the signal transducers - gp130 in the case of IL-6 (Murakami et al., Science 260:1808-1810 (1993) and IL-11, gp130 and LIFR in the case of CNTF (Davis et al., Science 260:1805-1808 (1993) - brings about signal transduction.

The initial heterodimeric molecules made comprised a soluble R α -component linked to the extracellular domain of gp130. These molecules

were shown to mimic the high affinity cytokine•R α •gp130 complex and behave as a high affinity antagonist of their cognate cytokine (Figure 7). To make these molecules, the extracellular domain of gp130 was paired with the extracellular domain of the α -receptor components for IL-6 and CNTF, IL-6R α 5 and CNTFR α respectively. To link the R α with the extracellular domain of gp130, the soluble R α -components and gp130 were fused to the Fc portion of human IgG1 to produce R α -Fc and gp130-Fc respectively. The Fc domain was chosen primarily but not solely because it naturally forms disulfide-linked dimers. Heterodimeric molecules comprising R α -Fc•gp130-Fc were 10 expressed, purified and shown to behave as highly potent antagonists of their cognate ligand. Furthermore, these molecules were found to be highly specific for their cognate cytokine since it is the choice of the α -receptor component which specifies which cytokine is bound and trapped (there is no measurable binding of the cytokine to gp130 in the absence of the appropriate 15 R α).

Here we describe an extension of this technology which allows the engineering of different heteromeric soluble receptor ligand traps which by virtue of their design may have additional beneficial characteristics such as stability, Fc-receptor-mediated clearance, or reduced effector functions (such as 20 complement fixation). Furthermore, the technology described should prove suitable for the engineering of any heteromeric protein in mammalian or other suitable protein expression systems, including but not limited to heteromeric molecules which employ receptors, ligands, and catalytic components such as enzymes or catalytic antibodies.

25 **MATERIALS AND METHODS**

Genetic Engineering of heteromeric immunoglobulin heavy/light chain soluble receptor-based ligand traps for IL-6.

The IL-6 traps described here were engineered using human gp130, human IL-6 α -receptor (IL-6R α), the constant region of the heavy chains ($C\gamma$) of human IgG1 ($C\gamma 1$) (Lewis et al., Journal of Immunology 151:2829-2838 (1993) or IgG4 ($C\gamma 4$) with or without a join-region (J), and the constant regions of 5 kappa (κ) and lambda (λ) (Cheung, et al., Journal of Virology 66:6714-6720 (1992) light chains of human immunoglobulin (Ig), also with or without a different j-peptide (j). This design takes advantage of the natural ability of the $C\gamma$ domain to heterodimerize with κ or λ light chains. The heterodimerization of $C\gamma$ with the light chain occurs between the CH1 10 domain of $C\gamma$ and the constant region of the light chain (CL), and is stabilized by covalent linking of the two domains via a single disulfide bridge. We reasoned that, like the Fc domain of human IgG1, the combination of $C\gamma$ with CL could be used to produce disulfide linked heteromeric proteins comprised 15 of the extracellular domain of gp130 on one chain and the extracellular domain of IL-6R α on the other chain. Like their Fc-based counterparts, such proteins were postulated to be high affinity ligand traps for IL-6 and as a result to inhibit the interaction of IL-6 with the native receptor on IL-6-responsive cells, thus functioning as IL-6 antagonists. Furthermore, constructs employing the full length $C\gamma$ region would, much like antibodies, form 20 homodimers of the $C\gamma$ chain, giving rise to antibody-like molecules comprising of two "light chains" and two "heavy chains" (figure 8). The potential advantage of this design is that it may more closely mimic the IL- 25 6•IL-6R α •gp130 complex and may display a higher affinity for the ligand than comparable single heterodimers. An additional design is incorporated by using truncated versions of $C\gamma$, comprised only of the CH1 domain. These will form heterodimeric molecules with receptor- κ fusion proteins, and will thus resemble the Fab fragment of antibodies.

All the soluble receptor-Ig chimeric genes may be engineered in plasmid vectors including, but not limited to, vectors suitable for mammalian expression (Cos monkey kidney cells, Chinese Hamster Ovary cells [CHO], and ras-transformed fibroblasts [MG-ras]) and include a Kozak sequence (CGC CGC CAC CAT GGT G) at the beginning of each chimeric gene for efficient translation. Engineering was performed using standard genetic engineering methodology. Each construct was verified by DNA sequencing, mammalian expression followed by western blotting with suitable antibodies, biophysical assays that determine ligand binding and dissociation, and by growth inhibition assays (XG-1, as described later). Since the domains utilized to engineer these chimeric proteins are flanked by appropriate restriction sites, it is possible to use these domains to engineer other chimeric proteins, including chimeras employing the extracellular domains of the receptors for factors such as IL-1, IL-2, IL-3, IL-4, IL-5, GM-CSF, LIF, IL-11, IL-15, IFN γ , TGF β , and others. The amino acid coordinates for each component utilized in making the IL-6 traps are listed below (Note: numbering starts with the initiating methionine as #1; long sequences are listed using the single letter code for the twenty amino acids):

(a) Constructs employing human gp130:

- (i) gp130-C γ 1 was engineered by fusing in frame the extracellular domain of gp130 (amino acids 1 to 619) to a Ser-Gly bridge, followed by the 330 amino acids which comprise C γ 1 and a termination codon (Figure 9).
- (ii) gp130-J-C γ 1 was engineered in the same manner as gp130-C γ 1 except that a J-peptide (amino acid sequence: GQGTLVTVSS) was inserted between the Ser-Gly bridge and the sequence of C γ 1 (see Figure 9).
- (iii) gp130 Δ 3fibro-C γ 1 was engineered by fusing in frame the extracellular domain of gp130 without its three fibronectin-like domains (Figure 10). The

remaining part of this chimeric protein is identical to gp130-C γ 1.

(iv) gp130-J-CH1 was engineered in a manner identical for that described for gp130-C γ 1, except that in place of the C γ 1 region only the CH1 part of C γ 1 has been used (Figure 11). The C-terminal domain of this construct includes the

5 part of the hinge that contains the cysteine residue responsible for heterodimerization of the heavy chain of IgG with a light chain. The part of the hinge that contains the two cysteines involved in C γ 1 homodimerization has been deleted along with the CH2 and CH3 domains.

(v) gp130-C γ 4 was engineered in a manner identical to that described for

10 gp130-C γ 1, except that C γ 4 was used in place of C γ 1 (Figure 12). In addition, an RsrII DNA restriction site was engineered at the hinge region of the C γ 4 domain by introducing two silent base mutations. The RsrII site allows for other desired genetic engineering manipulations, such as the construction of the CH1 equivalent of gp130-C γ 4.

15 (vi) gp130- κ was engineered in a manner identical to that described for gp130-C γ 1, except that the constant region of the κ light chain of human Ig was used in place of C γ 1 (Figure 13).

(vi) gp130-J- κ was engineered in a manner identical to that described for gp130-J- κ , except that a j-peptide (amino acid sequence: TFGQGTKVEIK) was 20 inserted between the Ser-Gly bridge and the κ -region.

(viii) gp130- λ was engineered in a manner identical to that described for gp130-C γ 1, except that the constant region of the λ light chain (Cheung, et al., Journal of Virology 66:6714-6720 (1992) of human Ig was used in place of C γ 1 (Figure 14).

25 **Constructs employing human IL-6Ra:**

(i) IL6R-C γ 1 was engineered by fusing in frame amino acids 1 to 358 of IL-6Ra (Yamasaki et al., Science 241:825-828 (1988), which comprise the extracellular

domain of IL-6R α (Figure 15), to an Ala-Gly bridge, followed by the 330 amino acids which comprise C γ 1 and a termination codon.

(ii) IL6R- κ was engineered as described for IL6R-C γ 1, except that the κ -domain (Figure 13) utilized for gp130- κ was used in place of C γ 1.

5 (iii) IL6R-j- κ was engineered as described for IL6R- κ except that the j-peptide described for gp130-j- κ was placed between the Ala-Gly bridge and the κ -domain.

(iv) Three additional constructs, IL6R313-C γ 1, IL6R313- κ , and IL6R313-j- κ , were engineered as using a truncated form of IL-6Ra comprised of amino 10 acids 1 to 313 (Figure 16). Each of these constructs were made by fusing in frame IL6R313 with a Thr-Gly bridge followed by the C γ 1, κ -, and j- κ -domains described above. These constructs were engineered in order to complement the gp130 Δ 3fibro-derived constructs.

Expression and purification of ligand traps

15 To produce covalently linked heterodimers of soluble gp130 and soluble IL-6R α , gp130-Ig chimeric proteins were co-expressed with appropriate IL-6R α -Ig chimeric proteins in complementing pairs. Co-expression was achieved by co-transfected the corresponding expression vectors into suitable mammalian cell lines, either stably or transiently. The resulting disulfide-linked heterodimers were purified from conditioned media by several 20 different methods, including but not limited to affinity chromatography on immobilized Protein A or Protein G, ligand-based affinity chromatography, ion exchange, and gel filtration.

25 An example of the type of methods used for purification of a heavy/light receptor fusion protein is as follows: gp130-C γ 1•IL-6R α - κ was expressed in COS cells by co-transfected two different vectors, encoding gp130-C γ 1 and IL-6R α - κ respectively. Serum-free conditioned media (400 ml) were collected two days post-transfection and C γ 1-

bearing proteins were purified by affinity chromatography over a 1ml Protein A Sepharose (Pharmacia). The material generated in this step was further purified by a second affinity chromatography step over a 1 ml NHS-activated Sepharose (Pharmacia) which was derivatized with recombinant human IL-6, in order to remove gp130-C γ 1 dimer from gp130-C γ 1•IL-6R α - κ complexes (the gp130-C γ 1 dimer does not bind IL-6). Proteins generated by this method were more than 90% pure, as evidenced by SDS-PAGE followed by silver-staining (Figure 17). Similar protocols have been employed successfully towards the purification of other heavy/light receptor heterodimers.

RESULTS

15 Biological activity of immunoglobulin heavy/light chain receptor fusion antagonists

The purified ligand traps were tested for their ability to bind IL-6 in a variety of different assays. For example, the dissociation rate of IL-6 bound to the ligand trap was measured in parallel with the dissociation rate of IL-6 from the anti-IL-6 monoclonal neutralizing antibody B-E8 [Brochier, et al., Int. J. Immunopharmacology 17:41-48 (1995), and references within]. An example of this type of experiment is shown In Figure 18. In this experiment 20 pM 125 I-IL-6 (1000 Ci/mmol; Amersham) was preincubated with 500 pM of either gp130-C γ 1•IL-6R α - κ or mAb B-E8 for 20 hours. At this point a 1000-fold excess (20 nM) of "cold" IL-6 was added. Periodically, aliquots of the reaction were removed, the ligand trap or B-E8 were precipitated with Protein G-Sepharose, and the number of cpm of 125 I-IL-6 that remained bound was determined. Clearly, the dissociation rate of human 125 I-IL6 from the ligand trap was very slow - after three days, approximately 75% of the initial counts were still bound to the ligand trap. In contrast, less than 5% of the counts remained associated with the antibody after three days. This result demonstrates that the dissociation rate of the ligand from these ligand traps is very slow.

In a different set of experiments the ability of the ligand traps to

multimerize in the presence of ligand was tested. An example of this is shown on Figure 19. IL-6-induced association of gp130-Fc•IL-6R α -Fc with gp130-CH1•IL-6R α - κ was determined by testing whether gp130-CH1•IL-6R α - κ , which does not by itself bind protein A, could be precipitated by protein A-5 Sepharose in the presence of gp130-Fc•IL-6R α -Fc in an IL-6-dependend manner (Figure 9). Precipitation of gp130-CH1•IL-6R α - κ by Protein A-Sepharose was determined by western blotting with an anti-kappa specific HRP conjugate, which does not detect gp130-Fc•IL-6R α -Fc. gp130-CH1•IL-6R α - κ could be precipitated by Protein A-Sepharose only when both gp130-Fc•IL-6R α -Fc and 10 IL-6 were present. This result conclusively indicates that IL-6 can induce ligand trap multimerization, and further indicate that the ligand trap can mimic the hexameric cytokine•R α •signal transducer complex (Figure 1). Ligand-induced multimerization may play a significant role in the clearance 15 of cytokine•ligand trap complexes *in vivo*.

The biological activity of the different ligand traps may be further tested in assays which measure ligand-dependend cell proliferation. Several cell proliferation assays exist for IL-6 and they employ cell lines such as B9, CESS, or XG-1. An example of this type of assay using the XG-1 cell line is presented below: XG-1 is a cell line derived from a human multiple myeloma (Zhang, 20 et al., Blood 83:3654-3663 (1994). XG-1 depends on exogenously supplied human IL-6 for survival and proliferation. The EC50 of IL-6 for the XG-1 line is approximately 50 pmoles/ml. The ability of several different IL-6 traps to block IL-6-dependend proliferation of XG-1 cells was tested by incubating increasing amounts of purified ligand traps with 50 pg/ml IL-6 in XG-1 25 cultures. The ligand traps which were tested had been expressed and purified by methods similar to those described above. All of the ligand traps tested were found to inhibit IL-6-dependent proliferation of XG-1 in a dose

dependent manner (Figure 20). Of the five different traps tested gp130-C γ 1•IL-6R α - κ was the most active and essentially display the same neutralizing activity towards IL-6 as the antibody B-E8. As little as a 10-fold molar excess of either gp130-C γ 1•IL-6R α - κ or B-E8 completely blocked the activity of IL- 6 (a reading of A570-650 = 0.3 AU corresponds to no proliferation of the XG-1 cells). At a 100-fold molar excess all of the ligand traps tested completely blocked the activity of IL-6. This observed inhibition is highly selective as neither a gp130-Fc•CNTFR α -Fc ligand trap which blocks CNTF activity, nor gp130-Fc homodimer exhibit any blocking activity towards IL-6 even when used at a 1000-fold molar excess over IL-6 (data not shown). This data demonstrates that the heteromeric immunoglobulin heavy/light chain receptor-based ligand traps function as selective high affinity antagonists of their cognate ligand.

15 **EXAMPLE 5 - CLONING OF FUSION POLYPEPTIDE COMPONENTS**

The extracellular domains of the human cytokine receptors were obtained by standard PCR techniques using tissue cDNAs (CLONTECH), cloned into the expression vector, pMT21 (Genetics Institute, Inc.), and the sequences were sequenced by standard techniques using an ABI 373A DNA sequencer and Taq Dideoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). For the IL-4Ra, nucleotides 241 through 868 (corresponding to the amino acids 24-231) from the Genbank sequence, X52425, were cloned. For the IL-2Rg, nucleotides 15 through 776 (corresponding to amino acids 1-233) from the Genbank sequence, D11086, were cloned. For the IL-6Ra, nucleotides 52 through 1044 (corresponding to the amino acids 1-331) from the Genbank sequence, X52425, were cloned. For gp130, nucleotides 322 through 2112 (corresponding to the amino acids 30-619) from the Genbank sequence, M57230, were cloned. For the IL-1RAcP, nucleotides 1 through 1074

(corresponding to the amino acids 1-358) from the Genbank sequence, AB006357, were cloned. For the IL-1RI, nucleotides 55 through 999 (corresponding to the amino acids 19-333) from the Genbank sequence, X16896, were cloned.

5

EXAMPLE 6 - PRODUCTION OF FUSION POLYPEPTIDES
(CYTOKINE TRAPS)

- 10 The nucleotide sequences encoding the cytokine traps were constructed from the individual cloned DNAs (described *supra*) by standard cloning and PCR techniques. In each case, the sequences were constructed in frame such that the sequence encoding the first fusion polypeptide component was fused to the sequence encoding the second fusion polypeptide component followed by an Fc domain (hinge, CH2 and CH3 region of human IgG1) as the multimerizing component. In some cases extra nucleotides were inserted in frame between sequences encoding the first and second fusion polypeptide components to add a linker region between the two components (See Figs. 21A-21D - trap 424; Figs. 24A-24F - trap 412; and Figs. 26A-26E - trap 569).
- 15
- 20 For the IL-4 traps, 424 (Figs. 21A-21D), 603 (Figs. 22A-22D) and 622 (Figs. 23A-23D), the IL-2R γ component is 5', followed by the IL4R α component and then the Fc component. For the IL-6 traps, 412 (Figs. 24A-24F) and 616 (Figs. 25A-25F), the IL-6R α component is 5' followed by the gp130 component and then the Fc domain. For the IL-1 trap 569 (Figs. 26A-26E), the IL-1RAcP component is 5' followed by the IL-1RI component and then the Fc domain. The final constructs were cloned into the mammalian expression vector pCDNA3.1 (STRATAGENE).
- 25

In the 569 sequence (Figs. 26A-26E), nucleotides 1-1074 encode the IL1RAcP component, nucleotides 1075 -1098 encode a linker region, nucleotides 1099-2043 encode the IL1RI component and nucleotides 2044-2730 encode the Fc domain.

5

In the 412 sequence (Figs. 24A-24F), nucleotides 1-993 encode the IL6R α component, nucleotides 994-1023 encode a linker region, nucleotides 1024-2814 encode the gp130 component and nucleotides 2815-3504 encode the Fc domain.

10

In the 616 sequence (Figs. 25A-25F), nucleotides 1-993 encode the IL6R α component, nucleotides 994-2784 encode the gp130 component and nucleotides 2785-3474 encode the Fc domain.

15

In the 424 (Figs. 21A-21D) and 622 (Figs. 23A-23D) sequences, nucleotides 1-762 encode the IL2R γ component, nucleotides 763-771 encode a linker region, nucleotides 772-1395 encode the IL4R α component and nucleotides 1396-2082 encode the Fc domain.

20

Finally, in the 603 sequence (Figs. 22A-22D), nucleotides 1-762 encode the IL2R γ component, nucleotides 763-1386 encode the IL4R α component and nucleotides 1387-2073 encode the Fc domain.

25

DNA constructs were either transiently transfected into COS cells or stably transfected into CHO cells by standard techniques well known to one of skill in the art. Supernatants were collected and purified by protein A affinity chromatography and size exclusion chromatography by standard techniques. (See for example Harlow and Lane, *Antibodies - A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988).

Example 7: IL-4 Bioassay Protocol using TF-1 (ATCC) cells.

5

Reagents and Equipment Needed

MTT Dye Solution:

MTT(3-[4,5-Dimethylthiazole-2-yl]) (Sigma catalog# M2128)

- 10 Working concentration: Dissolve 5 mg of anhydrous MTT in 200 ml PBS without Ca⁺², Mg⁺².
Sterile filter and store aliquoted at -20°C

Solubilization Solution:

- 15 For 1000 ml, combine 100 g SDS, 950 ml dH₂O, 50 ml Dimethyl Formamide, and 850 µl concentrated HCl.
Filter sterilize with a 0.45µm filter unit.
Store at room temperature
- 20 TF-1 cell Growth Medium: RPMI 1640, 10%FBS, Pen/Strep, 2mM L-glutamine

- Other: 0.4% Trypan Blue Stain, sterile tubes for dilutions, sterile 96 well cell culture plates (Falcon#3072), hemacytometer, centrifuge, ELISA plate reader, multichannel pipet for 15, 25, 50 and 100µl volume, sterile reagent reservoirs, sterile pipet tips, gloves.

Assay Protocol

30 A. Preparation of Assay plates

1. Prepare sterile 96 well tissue culture plates to contain 50µl of growth medium per well with various concentrations of IL-4 and 10nM IL-4 antagonist. This can be done by preparing a working dilution of IL-4 that is 4 times the highest concentration to be assayed. In separate tubes, do a two-fold

serial dilution of the IL-4. Add 25 μ l of each dilution to one row across the plate (i.e. row A gets highest concentration, row G gets lowest concentration). Add 25 μ l of growth medium without IL-4 to row H. Prepare the antagonists to be tested by making a stock that is 4 times the final concentration. Add

- 5 25 μ l to a triplicate set of IL-4 containing wells (columns 1,2,3, A through H). Be sure to include antagonist in row H.

10 2. As a positive control, leave one set with no antagonist. These wells will contain IL-4 and media only.

- 15 3. Incubate the plate for 1-2 hours at 37oC in a humidified 5% CO₂ incubator before preparing cells to be used for assay.

15 **B. Preparation of Cells**

- 20 4. Wash cells twice by centrifugation in assay medium free of growth factor.

- 25 5. Determine cell number and trypan blue viability and suspend cells to a final concentration of 8×10^5 /ml in assay medium.

- 20 6. Dispense 50 μ l of the cell suspension (40,000 cells) into all wells of the plates. Total volume should now be 100 μ l/well.

- 25 7. Incubate the plate at 37oC for 68 hours in a humidified 5% CO₂ incubator.

25 **C. Color Development**

- 30 8. After incubating for 68 hours, add 15 μ l of the MTT dye solution to each well.

- 30 9. Incubate the plate at 37oC for 4 hours in a humidified 5% CO₂ incubator.

- 35 10. After 4 hours, add 100 μ l of the solubilization solution to each well. Allow the plate to stand overnight in a sealed container to completely solubilize the formazan crystals.

11. Record the absorbance at 570/650nm.

Results:

5

Figure 27 shows that an IL-4 trap designated 4SC375, which is a fusion polypeptide of IL-2R γ -scb-IL4R α -Fc Δ C1, is several orders of magnitude better as an IL-4 antagonist than IL4R α Fc Δ C1 alone in the TF1 cell bioassay.

10

Figure 28 shows that the IL-4 trap designated 4SC375 shows antagonistic activity in the TF1 cell bioassay equivalent to an IL-4 trap designated 4SC424 which is a fusion polypeptide of IL-2R γ -IL4R α -Fc Δ C1 having the IL-2R γ component flush with the IL-4R α component.

15

Example 8: IL-6 Bioassay Protocol using XG-1 cells

Reagents and Equipment Needed

20

MTT Dye Solution:

MTT(3-[4,5-Dimethylthiazole-2-yl]) (Sigma catalog# M2128)

Working concentration: Dissolve 5 mg of anhydrous MTT in 200 ml PBS without Ca+2, Mg+2.

Sterile filter and store aliquoted at -20°C

25

Solubilization Solution:

For 1000 ml, combine 100 g SDS, 950 ml dH₂O, 50 ml Dimethyl Formamide, and 850 μ l concentrated HCl.

Filter sterilize with a 0.45 μ m filter unit.

30

Store at room temperature

Assay Medium: RPMI

1640, 10%FBS, Pen/Strep, 2mM L-glutamine, 50 μ M mercapto-ethanol.

Other: 0.4% Trypan Blue Stain, sterile tubes for dilutions, sterile 96 well cell culture plates (Falcon#3072), hemacytometer, centrifuge, ELISA plate reader, multichannel pipet for 15, 25, 50 and 100 μ l volume, sterile reagent reservoirs, 5 sterile pipet tips, gloves.

Assay Protocol

A. Preparation of Assay plates

10

1. Prepare sterile 96 well tissue culture plates to contain 50 μ l of growth medium per well with various concentrations of IL-6 and 10nM IL-6 antagonist. This can be done by preparing a working dilution of IL-6 that is 4 times the highest concentration to be assayed. In separate tubes, do a two-fold serial dilution of the IL-6. Add 25 μ l of each dilution to one row across the plate (i.e. row A gets highest concentration, row G gets lowest concentration). Add 25 μ l of growth medium without IL-6 to row H. Prepare the antagonists to be tested by making a stock that is 4 times the final concentration. Add 25 μ l to a triplicate set of IL-6 containing wells (columns 1,2,3, A through H). Be sure to include antagonist in row H. A typical IL-6 titration starts at 200ng/ml down to 3.1ng/ml.

2. As a positive control, leave one set with no antagonist. These wells contain IL-6 and media in place of antagonist.

25

3. Incubate the plate 1-2 hours at 37oC in a humidified 5% CO₂ incubator before preparing cells to be used for assay.

B. Preparation of Cells

30

4. Wash cells twice by centrifugation (5 min at 1000RPM) in assay medium free of growth factor.

35 5. Determine cell number and trypan blue viability and suspend cells to a final concentration of 8 x 10⁵/ml in assay medium.

6. Dispense 50 μ l of the cell suspension (40000 cells) into all wells of the plates. Total volume should now be 100 μ l/well.

7. Incubate the plate at 37°C for 68 hours in a humidified 5% CO₂ incubator.

5

C. Color Development

8. At 68 hours add 15 μ l of the dye solution to each well.

10 9. Incubate the plate at 37°C for 4 hours in a humidified 5% CO₂ incubator.

10. After 4 hours, add 100 μ l of the solubilization solution to each well. Allow the plate to stand overnight in a sealed container to completely solubilize the formazan crystals.

15

11. Record the absorbance at 570/650nm.

Results:

20 Figure 29 shows that the IL6 trap (6SC412 IL6R-scb-gpx-Fc Δ C1) described in Figs. 24A-24F is a better antagonist of IL-6 in the XG1 bioassay than the neutralizing monoclonal antibody to human IL-6 - BE8.

25 Example 9: MRC5 Bioassay for IL1 Traps

MRC5 human lung fibroblast cells respond to IL-1 by secreting IL-6 and thus were utilized to assay the ability of IL-1 traps to block the IL-1-dependent production of IL-6. IL1 Trap 1SC569 (Figs. 26A-26E) was tested against IL-1-RI.Fc which is the extracellular domain of the IL-1 Type I receptor fused to an Fc domain.

MRC5 cells are suspended at 1×10^5 cells per ml in medium and 0.1 ml of cells are plated (10,000 cells per well) into the wells of a 96 well tissue culture plate. Plates are incubated for 24 hours at 37°C in a humidified 5% CO₂ incubator.

5

IL-1 trap and recombinant human IL-1 at varying doses are pre-incubated in a 96 well tissue culture dish and incubated for 2 hours at 37°C. 0.1 ml of this mixture is then added to the 96 well plate containing the MRC5 cells such that the final concentration of IL-1 Trap is 10nM and the final concentrations of 10 the IL-1 ranges from 2.4 pM to 5nM. Control wells contain trap alone or nothing.

Plates are then incubated at 37°C for 24 hours in a humidified 5% CO₂ incubator. Supernatant is collected and assayed for levels of IL-6 using R&D 15 Systems Quantikine Immunoassay Kit according to the manufacturer's instructions.

Results:

Figure 30 shows that the trap 569 (Figs. 26A-26E) is able to antagonize the 20 effects of IL-1 and block the IL-6 production from MRC 5 cells upon treatment with IL-1. At a concentration of 10nM, the trap 569 is able to block the production of IL-6 up to an IL-1 concentration of 3nM. In contrast, the IL-1RI.Fc is a much poorer antagonist of IL-1. It is only able to block the effects of 25 IL-1 up to about 10-20 pM. Thus, the trap 569 is approximately 100x better at blocking IL-1 than IL1RI.Fc.

Example 10 - Construction of IL-13/IL-4 single chain traps

1. PCR cloning of human IL-13R α 1 from human spleen cDNA.

The extracellular domain of human IL-13R α 1 (corresponding to 5 nucleotides #34-1062 from Genbank Accession # Y09328) was amplified by standard PCR techniques. The PCR product was then ligated into an expression vector (pCDNA3.1 Stratagene) containing human Fc construct to create a fusion protein consisting of the extracellular domain of human IL-13R α 1 fused in frame with the hinge, CH2 and CH3 region of human IgG1.
10 The human IL-13R α 1 sequence was verified. The DNA encoding IL-13R α 1.Fc was transfected into COS cells and protein expression was verified by Western Blot analysis of the cell media.

15 2. Construction of IL-13/IL-4 Traps

a. IL-4R α .IL-13R α 1.Fc

The IL-13R α 1 extracellular domain (not including the predicted signal peptide, therefore nt #100-1062; amino acid #23-343) was amplified by 20 standard PCR techniques and ligated into an expression vector, pMT21, to create an in frame fusion downstream of the extracellular domain of human IL-4R α (nucleotides 176 through 868 (corresponding to the amino acids 1-231) from the Genbank sequence X52425) and upstream of the human Fc. The IL-4R α contains a nucleotide change at nt# 794 (T to A) to change the amino acid at #207 to a Ser from a Cys. A two amino acid linker with the amino acid 25 sequence SG was constructed in frame between the IL-4R α and the IL-13R α 1. The human IL-13R α 1 sequence was verified. The construct encoding this single chain trap was transfected into COS cells and protein expression was verified by Western Blot analysis of the cell media. The nucleotide and

amino acid sequence of the IL-4R α .IL-13R α 1.Fc single chain trap is set forth in Figure 31A-31G.

b. IL-13R α 1.IL-4R α .Fc

- 5 The IL-13R α 1 extracellular domain (including the signal peptide, therefore
nts #34-1062; amino acid #1-343) was amplified by standard PCR techniques
and ligated into an expression vector, pJFE14, to create an in-frame fusion
upstream of the human IL-4Ra (nucleotides 236 through 868 (corresponding
to the amino acids 21-231) from the Genbank sequence X52425) and human
10 Fc. A ten amino acid linker with the amino acid sequence GAPSGGGGRP
was constructed in frame between the IL-4R α and the IL-13R α 1. The human
IL-13R α 1 sequence was verified. The construct encoding this single chain trap
was transfected into COS cells and protein expression was verified by Western
Blot analysis of the cell media. The nucleotide and amino acid sequence of the
15 IL-13R α 1.IL-4R α .Fc single chain trap is set forth in Figure 32A-32G.

The present invention is not to be limited in scope by the specific
embodiments described herein. Indeed, various modifications of the
20 invention in addition to those described herein will become apparent to those
skilled in the art from the foregoing description and accompanying figures.
Such modifications are intended to fall within the scope of the appended
claims.

WE CLAIM:

1. An isolated nucleic acid molecule encoding a fusion polypeptide capable of binding a cytokine to form a nonfunctional complex comprising:
 - 5 a) a nucleotide sequence encoding a first fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the specificity determining component of the cytokine's receptor;
 - b) a nucleotide sequence encoding a second fusion polypeptide component comprising the amino acid sequence of the cytokine binding portion of the extracellular domain of the signal transducing component of the cytokine's receptor; and
 - 10 c) a nucleotide sequence encoding a third fusion polypeptide component comprising the amino acid sequence of a multimerizing component.
- 15
- 20 2. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is upstream of the nucleotide sequence encoding the second component.
3. The nucleic acid molecule of claim 1, wherein the nucleotide sequence encoding the first component is downstream of the nucleotide sequence encoding the second component.
- 25 4. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the hematopoietin family of cytokines selected from the group consisting of interleukin-2, interleukin-3, interleukin-4, interleukin-5, interleukin-6, interleukin-7, interleukin-9, interleukin-11, interleukin-13, interleukin-15, granulocyte macrophage colony stimulating factor, oncostatin M, and leukemia inhibitory factor.
- 30

5. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the interferon family of cytokines selected from the group consisting of IFN-gamma, IFN-alpha, and IFN-beta.

5

6. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the immunoglobulin superfamily of cytokines selected from the group consisting of B7.1 (CD80) and B7.2 (B70).

10 7. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TNF family of cytokines selected from the group consisting of TNF-alpha, TNF-beta, LT-beta, CD40 ligand, Fas ligand, CD 27 ligand, CD 30 ligand, and 4-1BBL.

15 8. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a member of the TGF- β /BMP family selected from the group consisting of TGF- β 1, TGF- β 2, TGF- β 3, BMP-2, BMP-3a, BMP-3b, BMP-4, BMP-5, BMP-6, BMP-7, BMP-8a, BMP-8b, BMP-9, BMP-10, BMP-11, BMP-15, BMP-16, endometrial bleeding associated factor (EBAF), growth 20 differentiation factor-1 (GDF-1), GDF-2, GDF-3, GDF-5, GDF-6, GDF-7, GDF-8, GDF-9, GDF-12, GDF-14, mullerian inhibiting substance (MIS), activin-1, activin-2, activin-3, activin-4, and activin-5.

25 9. The isolated nucleic acid molecule of claim 1, wherein the cytokine receptor is the receptor for a cytokine selected from the group consisting of interleukin-1, interleukin-10, interleukin-12, interleukin-14 and MIF.

10. The isolated nucleic acid molecule of claim 1, wherein the multimerizing component comprises an immunoglobulin derived domain.

30

11. The isolated nucleic acid molecule of claim 10, wherein the immunoglobulin derived domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

5 12. A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 1.

13. A composition capable of binding a cytokine to form a nonfunctional complex comprising a multimer of the fusion polypeptide of claim 12.

10

14. The composition of claim 13, wherein the multimer is a dimer.

15. A vector which comprises the nucleic acid molecule of claim 1.

15 16. An expression vector comprising a nucleic acid molecule of claim 1, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

20 17. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 16, in a suitable host cell.

18. The host-vector system of claim 17, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell, or mammalian cell.

25 19. The host-vector system of claim 17, wherein the suitable host cell is E. coli.

20. The host-vector system of claim 17, wherein the suitable host cell is a COS cell.

30

21. The host-vector system of claim 17, wherein the suitable host cell is a CHO cell.

22. The host-vector system of claim 17, wherein the suitable host cell is a
5 293 cell.

23. The host-vector system of claim 17, wherein the suitable host cell is a BHK cell.

10 24. The host-vector system of claim 17, wherein the suitable host cell is a NS0 cell.

15 25. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 17, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced.

ABSTRACT

The present invention provides a fusion polypeptide capable of binding a
5 cytokine to form a nonfunctional complex. It also provides a nucleic acid
sequence encoding the fusion polypeptide and methods of making and uses
for the fusion polypeptide.

1/69
FIGURE 1

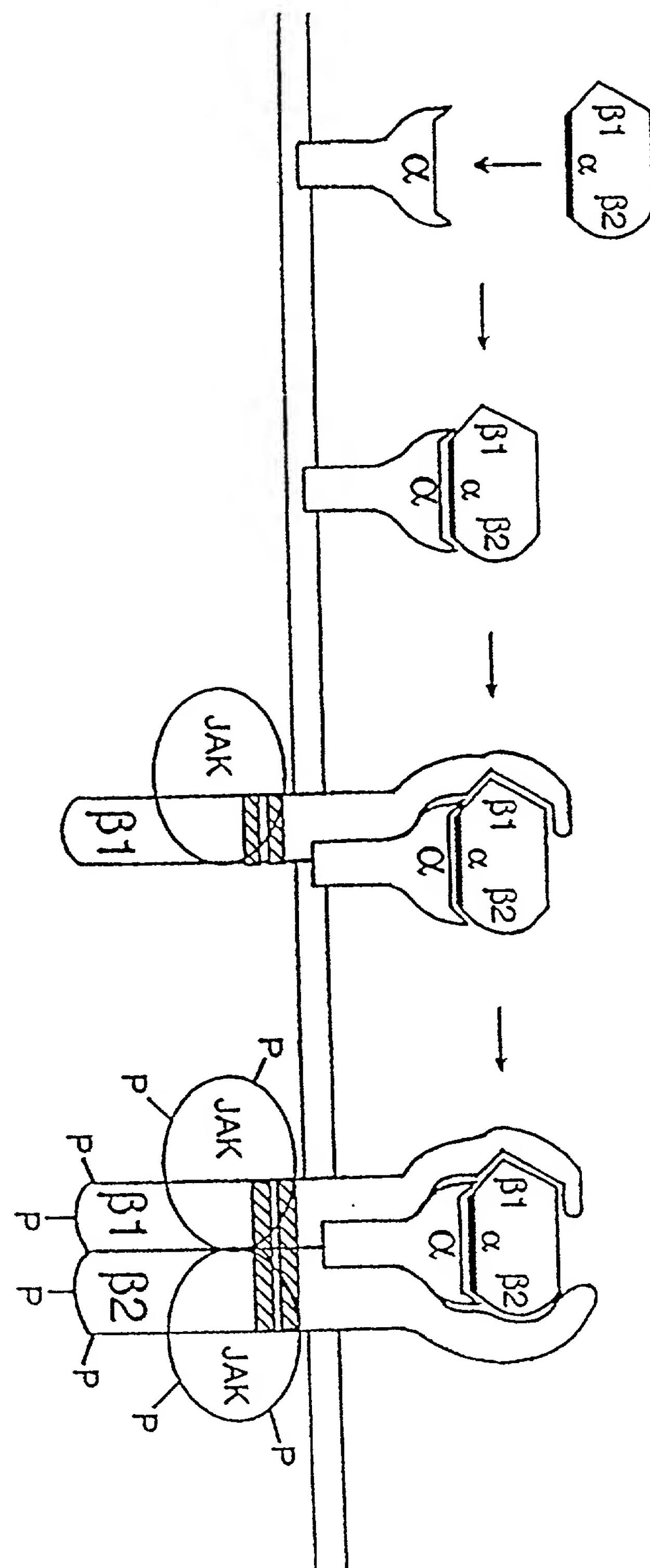
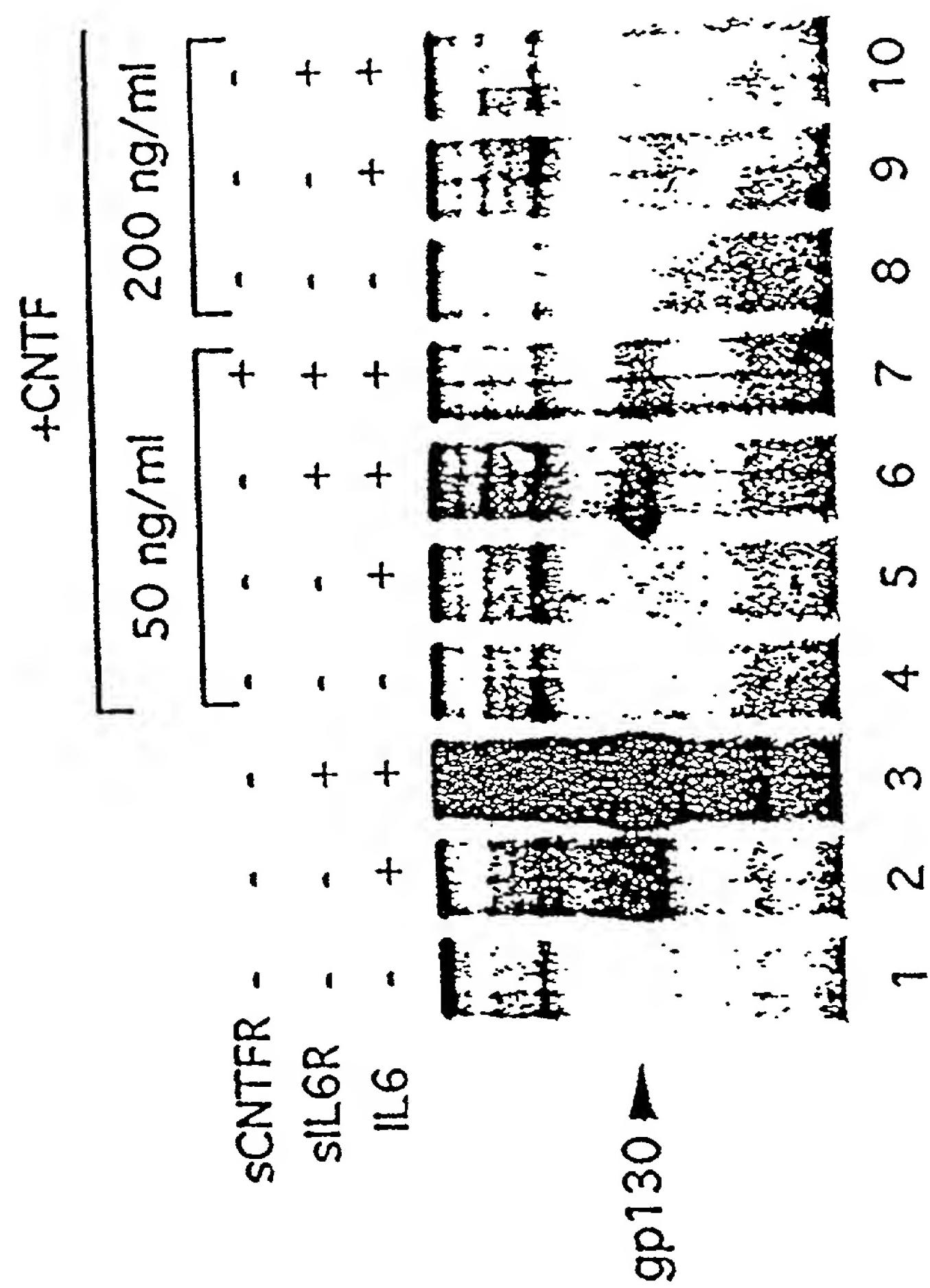


FIGURE 2



3/69
FIGURE 3

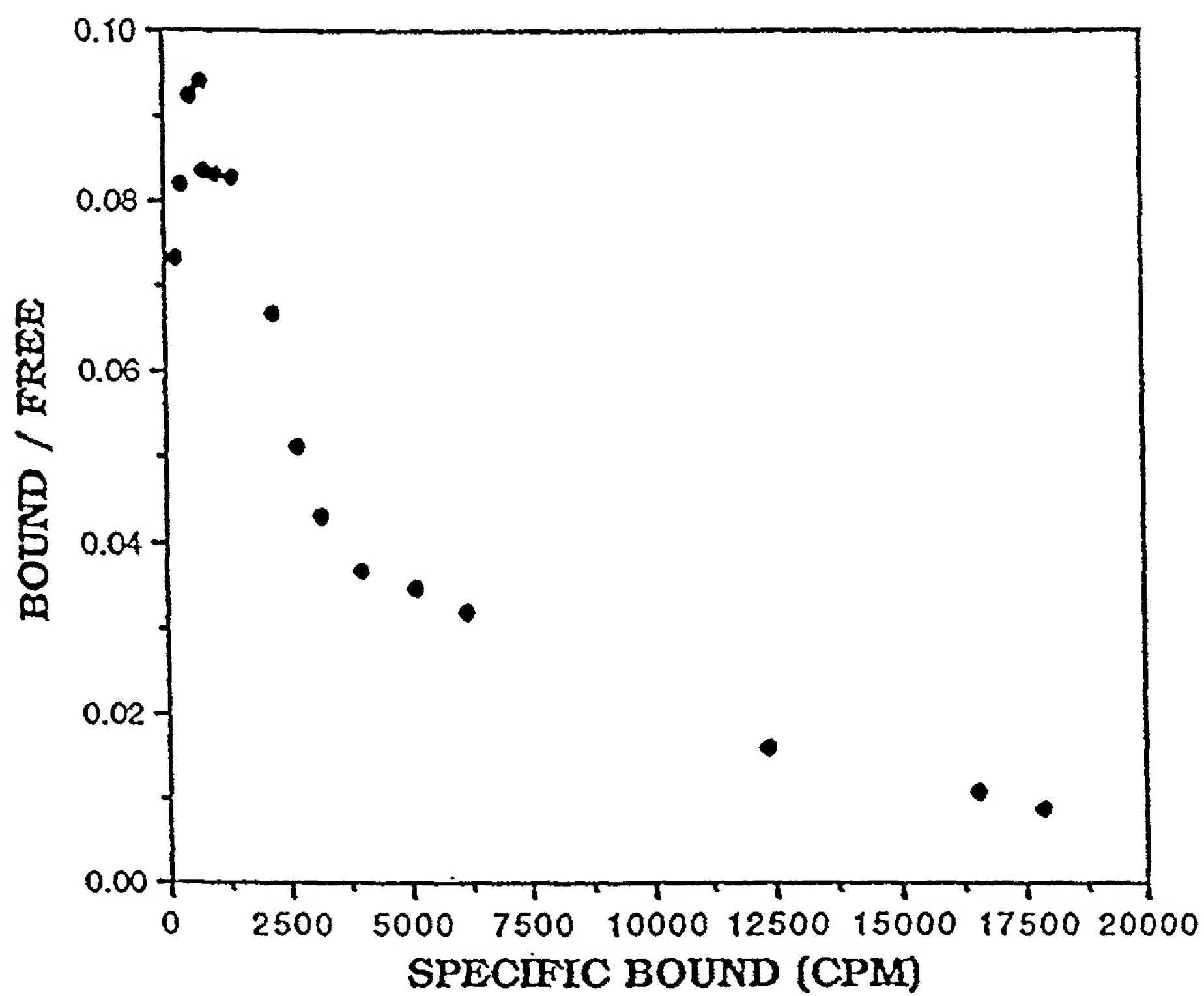


Figure 4

Amino acid sequence of human gp130-Fc-His6

Sequence Range: 1 to 861

10	20	30	40	50	60
*	*	*	*	*	*
MVTLQTWVVQALFIFLTTE S TGELLDPCGYISPEPSPVQL HSNFTAVCVLKEKCMDYFHV					
70	80	90	100	110	120
*	*	*	*	*	*
NANYIVWKTNHFTIPKEQYT IINRTASSVTFTDIASLNIQ LTCNILTGFQLEQNRYGITI					
130	140	150	160	170	180
*	*	*	*	*	*
ISGLPPEPKPNLSCIVNEGK KMRCEWDGGRETHLETNFTL KSEWATHKFADCKAKRDTPT					
190	200	210	220	230	240
*	*	*	*	*	*
SCTVDYSTVYFVNIEVWVEA ENALGKVTSVDHINFDPVYKV KPNPPHNLSVINSEELSSIL					
250	260	270	280	290	300
*	*	*	*	*	*
KLTWTNPSIKSVIILKYNIQ YRTKDASTWSQIPPEDTAST RSSFTVQDLKPFTEYVFRIR					
310	320	330	340	350	360
*	*	*	*	*	*
CMKEDGKGYWSDWSEEASGI TYEDRPSKAPSFWYKIDPSH TQGYRTVQLVWKTLPFEEAN					
370	380	390	400	410	420
*	*	*	*	*	*
GKILDYEVTLTRWKSHLQNY TVNATKLTVNLTNDRYLATL TVRNLVGKSDAAVLTIACD					
430	440	450	460	470	480
*	*	*	*	*	*
FQATHPVMDLKAFPKDNMLW VEWTTPRESVKKYILEWCVL SDKAPCITDWQQEDGTVHRT					
490	500	510	520	530	540
*	*	*	*	*	*
YLRGNLAESKCYLITVTPVY ADGPGSPESIKAYLKQAPPS KGPTVRTKKVGKNEAVLEWD					
550	560	570	580	590	600
*	*	*	*	*	*
QLPVDVQNGFIRNYTIFYRT IIGNETAVNVDSSSHTEYTLS SLTSDTLYMVRMAAYTDEGG					
610	620	630	640	650	660
*	*	*	*	*	*
KGDPFETFTTPKFAQGEIES GEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMIS					
670	680	690	700	710	720
*	*	*	*	*	*
RTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWL					
730	740	750	760	770	780
*	*	*	*	*	*

FIGURE 4 continued

NGKEYKCKVSNKALPAPIEK TISKAKGOPREPOVYTLPPS RDELTKNOVSLTCLVKGFYP

790 800 810 820 830 840
* * * * *

SDIAVEWESNGOPENNYKTT PPVLDSDGSSFLYSKLTVDK SRWOOGNVFSCSVMHEALHN

850 860
* *

HYTOKSLSLSPGKHHHHHH•

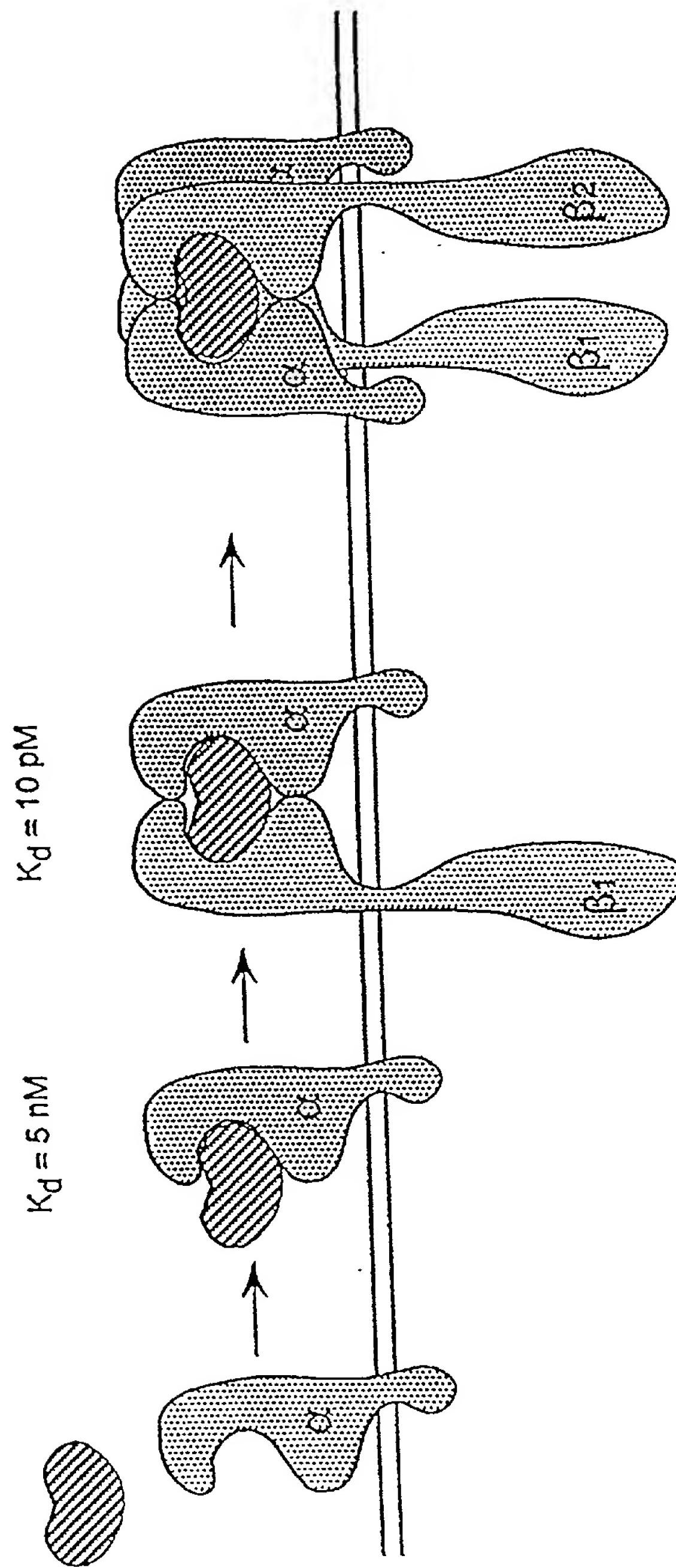
FIGURE 5

The amino acid sequence of human IL-6R α -Fc

Sequence Range: 1 to 594

10	20	30	40	50	60
*	*	*	*	*	*
MVAVGCALLAALLAAPGAAL APRRCPAQEVARVGLTSLPG DSVTLTCPGVEPEDNATVHW					
70	80	90	100	110	120
*	*	*	*	*	*
VLRKPAAGSHPSRWAGMGRRLLLRSVQLHDSGNYSCYRAG RPAGTVHLLVDVPPEEPQLS					
130	140	150	160	170	180
*	*	*	*	*	*
CFRKSPPLSNVVCEWGPRSTP SLTTKAVLLVRKFQNSPAED FQEPCQYSQESQKFSCQLAV					
190	200	210	220	230	240
*	*	*	*	*	*
PEGDSSFYIVSMCVASSVGS KFSKTQTFQCGILQPDPPA NITVTAVARNPRWLSVTWQD					
250	260	270	280	290	300
*	*	*	*	*	*
PHSWNSSFYRLRFELRYRAE RSKTFTTWMVKDLQHHCVIH DAWSGLRHVQLRAQEEFGQ					
310	320	330	340	350	360
*	*	*	*	*	*
GEWSEWSPEAMGTPWTESRS PPAENEVSTPMQALTINKDD DNILFRDSANATSLPVQDAG					
370	380	390	400	410	420
*†	†	*	*	*	*
<u>EPKSCDKTHCPPCPAPELL GGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKE</u>					
430	440	450	460	470	480
*	*	*	*	*	*
<u>NWYVDGVEVHNAAKTKPREEQ YNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKT</u>					
490	500	510	520	530	540
*	*	*	*	*	*
<u>ISKAKGOPREPOVYTLPPSR DELTKNOVSLTCLVKGFYPS DIAVEWESNGOPENNYKTTP</u>					
550	560	570	580	590	
*	*	*	*	*	
<u>PVLDSDGSEFLYSKLTVDKS RWOOGNVFCSVVMHEALHNHYTOKSLSLSPGK.</u>					

FIGURE . 6



Heterodimeric Receptor-Based Ligand Trap

FIGURE 7C

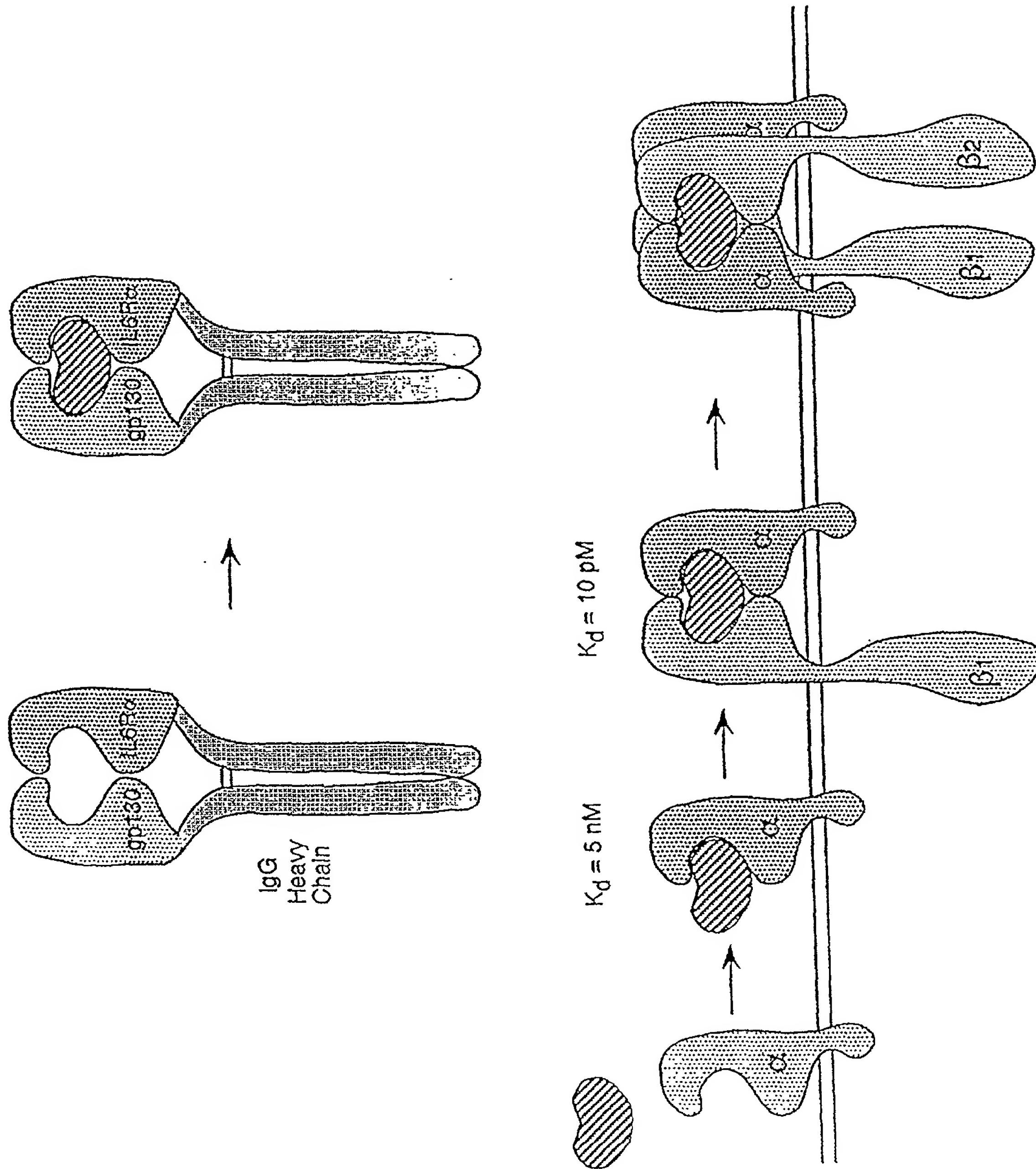
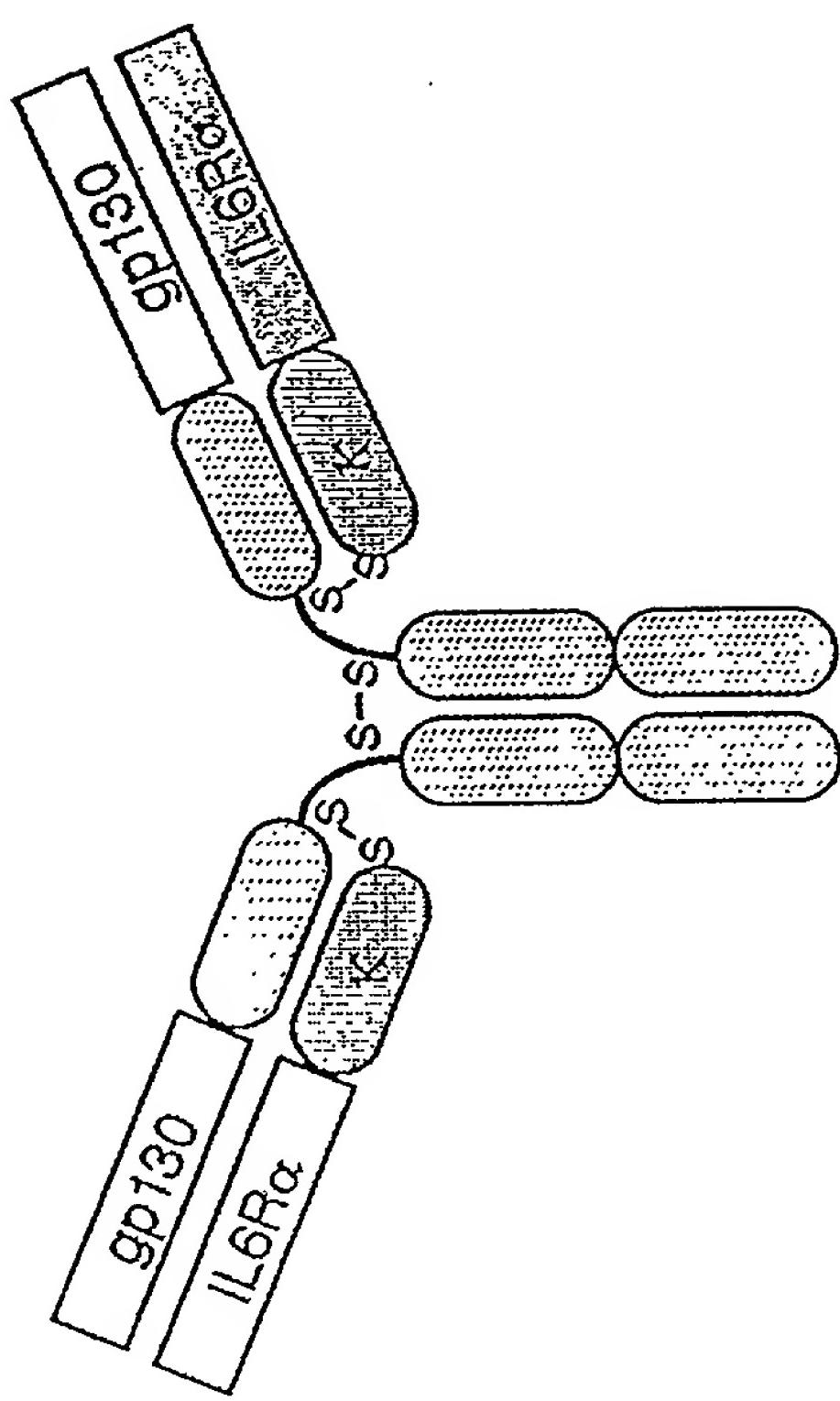


FIGURE 8.**Immunoglobulin Heavy/Light Chain Receptor Fusions**

10/69
FIGURE 9

Amino acid sequence of gp130-C γ 1

Sequence Range: 1 to 952

10	20	30	40	50	60
*	*	*	*	*	*
MVTLQTWVVQALFIFLTES TGELLDPCGYISPESPVVQL HSNFTAVCVLKEKCMDYFHV					
70	80	90	100	110	120
*	*	*	*	*	*
NANYIVWKTNHFTIPKEQYT IINRTASSVTFTDIASLNHQ LTCNILTGFQLEQNHYGITH					
130	140	150	160	170	180
*	*	*	*	*	*
ISGLPPEKPKNLSCIVNEGK KMRCEWDGGRETHLETNFTL KSEWATHKFADCKAKRDTPT					
190	200	210	220	230	240
*	*	*	*	*	*
SCTVDYSTVYFVNIEVVVEA ENALGKVTSVDHINFDPVYKV KPNPPHNLSVINSEELSSIL					
250	260	270	280	290	300
*	*	*	*	*	*
KLTWTNPSIKSVIILKYNHQ YRTKDASTWSQIIPPEDTAST RSSFTVQDLKPTEYVFRIR					
310	320	330	340	350	360
*	*	*	*	*	*
CMKEDGKGWSDWSEEASGI TYEDRPSKAPSFWYKIDPSH TQGYRTVQLVWKTLPFEEAN					
370	380	390	400	410	420
*	*	*	*	*	*
GKILDYEVTLTRWKSHLQNY TVNATKLTNVNLTNDRYLATL TVRNLVGKSDAAVLTIACD					
430	440	450	460	470	480
*	*	*	*	*	*
FQATHPVMDLKAFPKDNMLW VEWTTPRESVKYILEWCVL SDKAPCITDWQQEDGTVHRT					
490	500	510	520	530	540
*	*	*	*	*	*
YLRGNLAESKCYLITVTPVY ADGPGSPESIKAYLKQAPPS KGPTVRTKKVKGKNEAVLEWD					
550	560	570	580	590	600
*	*	*	*	*	*
QLPVDVQNGFIRNYTIFYRT IIGNETAVNVDSSSHTEYTLS SLTSDTLYMVRMAAYTDEGG					
610	620	630	640	650	660
*	*	*	*	*	*
KDGPEFTFTPKFAQGEIES GASTKGPSVPLAPSSKSTS GGTAAALGCLVKDYFPEPVTV					
670	680	690	700	710	720
*	*	*	*	*	*
<u>SWNSGALTSGVHTFPALVLOS SGLYSLSSVVTVPSSSLGTQ TYICNVNHKPSNTKVDKKVE</u>					
730	740	750	760	770	780
*	*	*	*	*	*
<u>PKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFN</u>					

11/69

FIGURE 9 continued

790
*

80
*

810
*

820
*

83
*

840
*

WYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHODWLNG KEYKCKVSNKALPAPIEKTI

850
*

860
*

870
*

880
*

890
*

900
*

SKAKGQPREGPOVYTLPPSRD ELTKNQVSLTCLVKGFYPSD IAVEWESNGOPENNYKTTPP

910
*

920
*

930
*

940
*

950
*

VLDSDGSFFLYSKLTVDKSR WOOGNVFSCSVMHEALHNHY TOKSLSLSPGK*

FIGURE 1.0

Amino acid sequence of gp130Δ3fibro

Sequence Range: 1 to 332

10 *	20 *	30 *	40 *	50 *	60 *
MVTLQTWVVQALFIFLTES TGELLDPCGYISPESPVQL HSNFTAVCVLKEKCMDYFHV					
70 *	80 *	90 *	100 *	110 *	120 *
NANYIVWKTNHFTIPKEQYT IINRTASSVTFTDIASLNIQ LTCNILTFGQLEQNIVYGITI					
130 *	140 *	150 *	160 *	170 *	180 *
ISGLPPEKPKNLSCIVNEGK KMRCEWDGGRETHLETNFTL KSEWATHKFADCKAKRDTPT					
190 *	200 *	210 *	220 *	230 *	240 *
SCTVDYSTVYFVNIEVVVEA ENALGKVTSDHINFDPVYKV KPNPPHNLHSVINSEELSSIL					
250 *	260 *	270 *	280 *	290 *	300 *
KLTWTNPSIKSVIILKYNIQ YRTKDASTWSQIPPEDTAST RSSFTVQDLKPTEYVFRIR					
310 *	320 *	330 *			
CMKEDGKGWSDWSEEASGI TYEDRPSKAPSG					

FIGURE 11

Amino acid sequence of J-CH1

Sequence Range: 1 to 121

10	20	30	40	50	60
*	*	*	*	*	*

SGGQGTLVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLOSSGLYSLSSV VTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHT*

70	80	90	100	110	120
*	*	*	*	*	*

FIGURE 12
Amino acid sequence of C γ 4

Sequence Range: 1 to 330

10	20	30	40	50	60
*	*	*	*	*	*
SGASTKGPSVFPLAPCSRST SESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPALQ					
70	80	90	100	110	120
*	*	*	*	*	*
SSGLYSLSSVVTVPSSSLGT KTYTCNVDHKPSNTKVDKRV ESKYGPPCPSCPAPAEFLGGP					
130	140	150	160	170	180
*	*	*	*	*	*
SVFLFPPPKPKDTLMISRTPE VTCVVVDVSQEDPEVQFNWY VDGVEVHNAAKTPREEQFNS					
190	200	210	220	230	240
*	*	*	*	*	*
TYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEM					
250	260	270	280	290	300
*	*	*	*	*	*
TKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTPPPVL DSDGSFFLYSRLTVDKSRWQ					
310	320	330			
*	*	*			
EGNVFSCSVMHEALHNHYTQ KSLSLSLGK*					

FIGURE 13

Amino acid sequence of κ-domain

Sequence Range: 1 to 108

10	20	30	40	50	60
*	*	*	*	*	*
SGTVAAPSVFIFPPSDEQLK SGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQ					
70	80	90	100		
*	*	*	*		
DSKDSTYSLSSTLTLSKADY EKHKVYACEVTHQGLSSPVT KSFNRGEC*					

FIGURE 14
Amino acid sequence of λ -domain:

Sequence Range: 1 to 107

10	20	30	40	50	60
*	*	*	*	*	*
SGPKAAPSVTLFPPSSEELQ ANKATLVCLISDFYPGAVTV AWKADSSPVKAGVETTPSK					
70	80	90	100		
*	*	*	*		
QSNNKYAASSYLSLTPEQWK SHRSYSCQVTHEGSTVEKTV APTECS*					

FIGURE 15

Amino acid sequence of the soluble IL-6R α domain

Sequence Range: 1 to 360

10	20	30	40	50	60
*	*	*	*	*	*
MVAVGCALLAALLAAPGAAL APRRCPAQEVARGVLTSLPG DSVTLTCPGVEPEDNATVHW					
70	80	90	100	110	120
*	*	*	*	*	*
VLRKPAAGSHPSRWAGMGR LLLRSVQLHDSGNYSCYRAG RPAGTVHLLVDVPPEEPQLS					
130	140	150	160	170	180
*	*	*	*	*	*
CFRKSPLSNVVCEWGPRSTP SLTTKAVLLVRKFQNSPAED FQEPCQYSQESQKFSCQLAV					
190	200	210	220	230	240
*	*	*	*	*	*
PEGDSSFYIVSMCVASSVGS KFSKTQTFQGCGILQPDPPA NITVTAVARNPRWLSVTWQD					
250	260	270	280	290	300
*	*	*	*	*	*
PHSWNSSFYRLRFELRYRAE RSKTFTTWMVKDLQHHCVIH DAWSGLRHVVQLRAQEEFGQ					
310	320	330	340	350	360
*	*	*	*	*	*
GEWSEWSPEAMGTPWTESRS PPAENEVSTPMQALTTNKDD DNILFRDSANATSLPVQDAG					

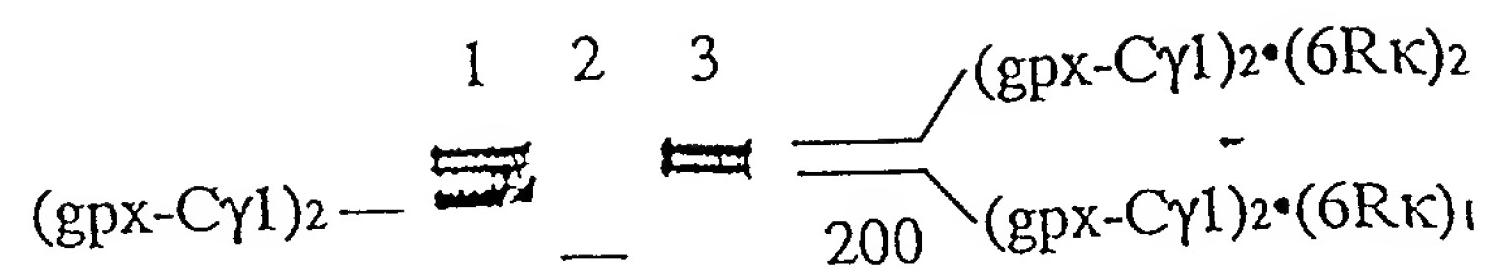
FIGURE 16

Amino acid sequence of the soluble IL-6 κ 313 domain

Sequence Range: 1 to 315

10	20	30	40	50	60
*	*	*	*	*	*
MVAVGCALLAALLAAPGAAL APRRCPAQEVARGVLTSLPG DSVTLTCPGVEPEDNATVHW					
70	80	90	100	110	120
*	*	*	*	*	*
VLRKPAAGSHPSRWAGMGRR LLLRSVQLHDSGNYSCYRAG RPAGTVHLLVDVPPEEPQLS					
130	140	150	160	170	180
*	*	*	*	*	*
CFRKSPLSNVVCEWGPRSTP SLTTKAVLLVRKFQNSPAED FQEPCQYSQESQKFSCQLAV					
190	200	210	220	230	240
*	*	*	*	*	*
PEGDSSFYIVSMCVASSVGS KFSKTQTFQGCGILQPDPPA NITVTAVARNPRWLSVTWQD					
250	260	270	280	290	300
*	*	*	*	*	*
PHSWNSSFYRLRFELRYRAE RSKTFTTWMVKDLQHHCVIH DAWSGLRHVQLRAQEEFGQ					
310					
*					
GEWSEWSPEAMGTTG					

FIGURE 17

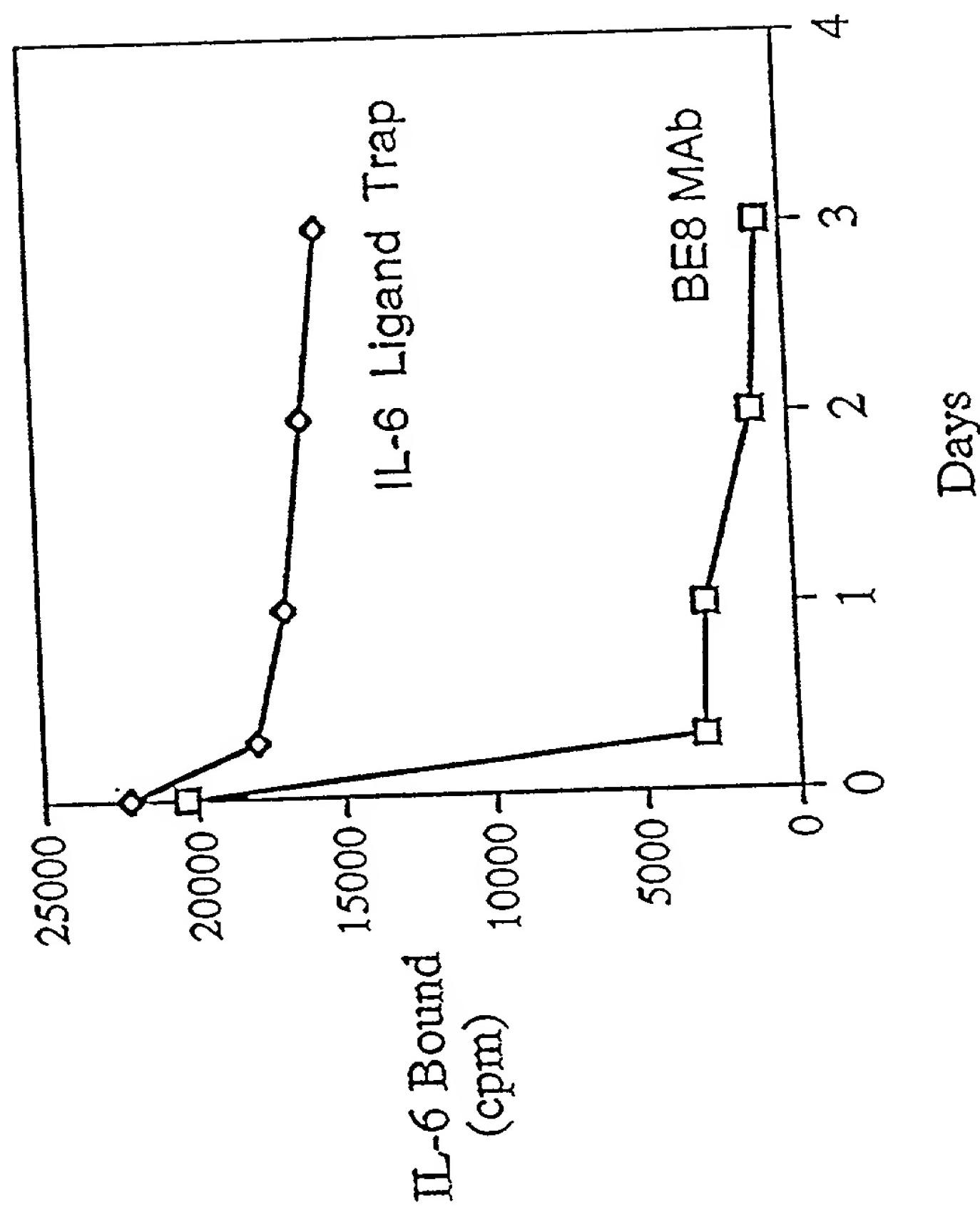


100

46

FIGURE 18

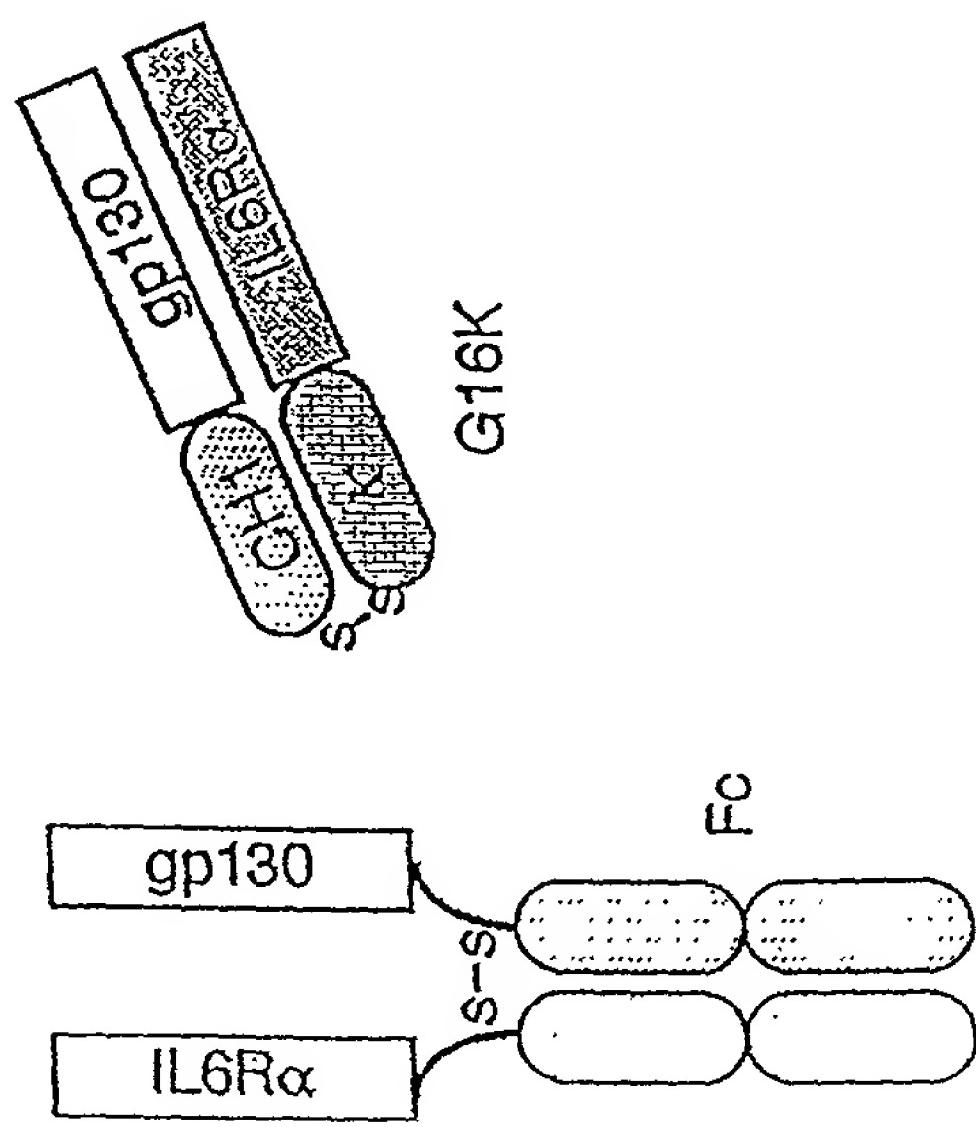
IL-6 Dissociates Slowly from the Ligand Trap



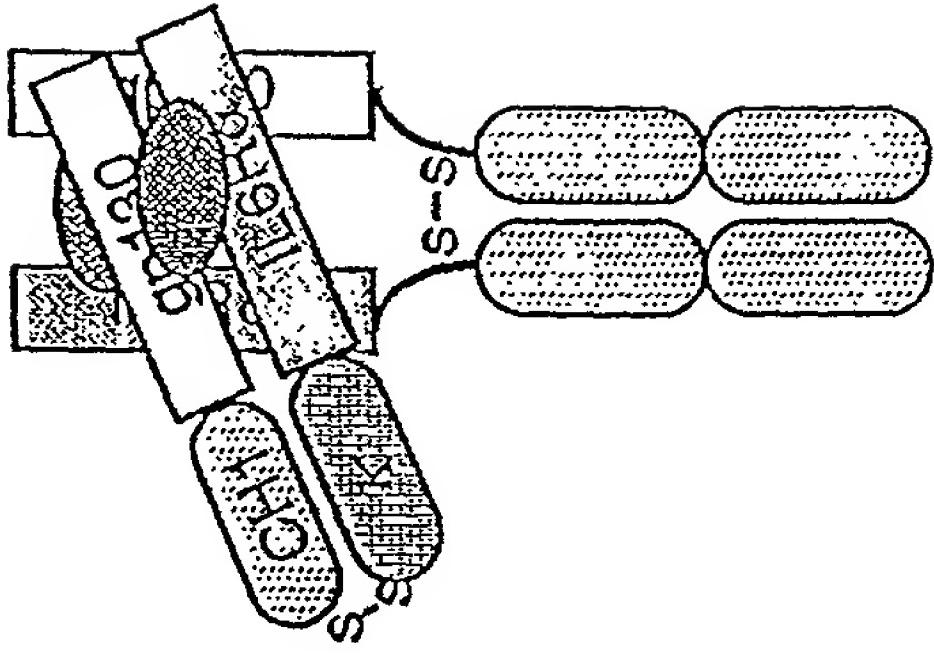
Does IL-6 Induce Complex Formation?

FIGURE 19

Protein A binding

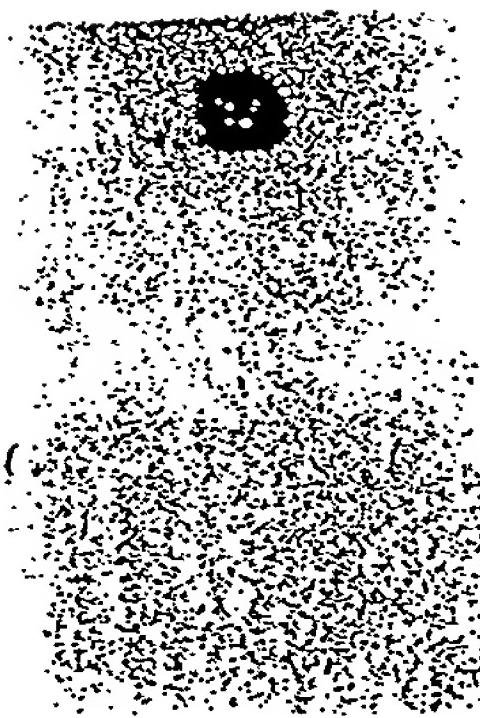


No Protein A binding



GF6F
&
G16K

IL-6: [-] + - + - +

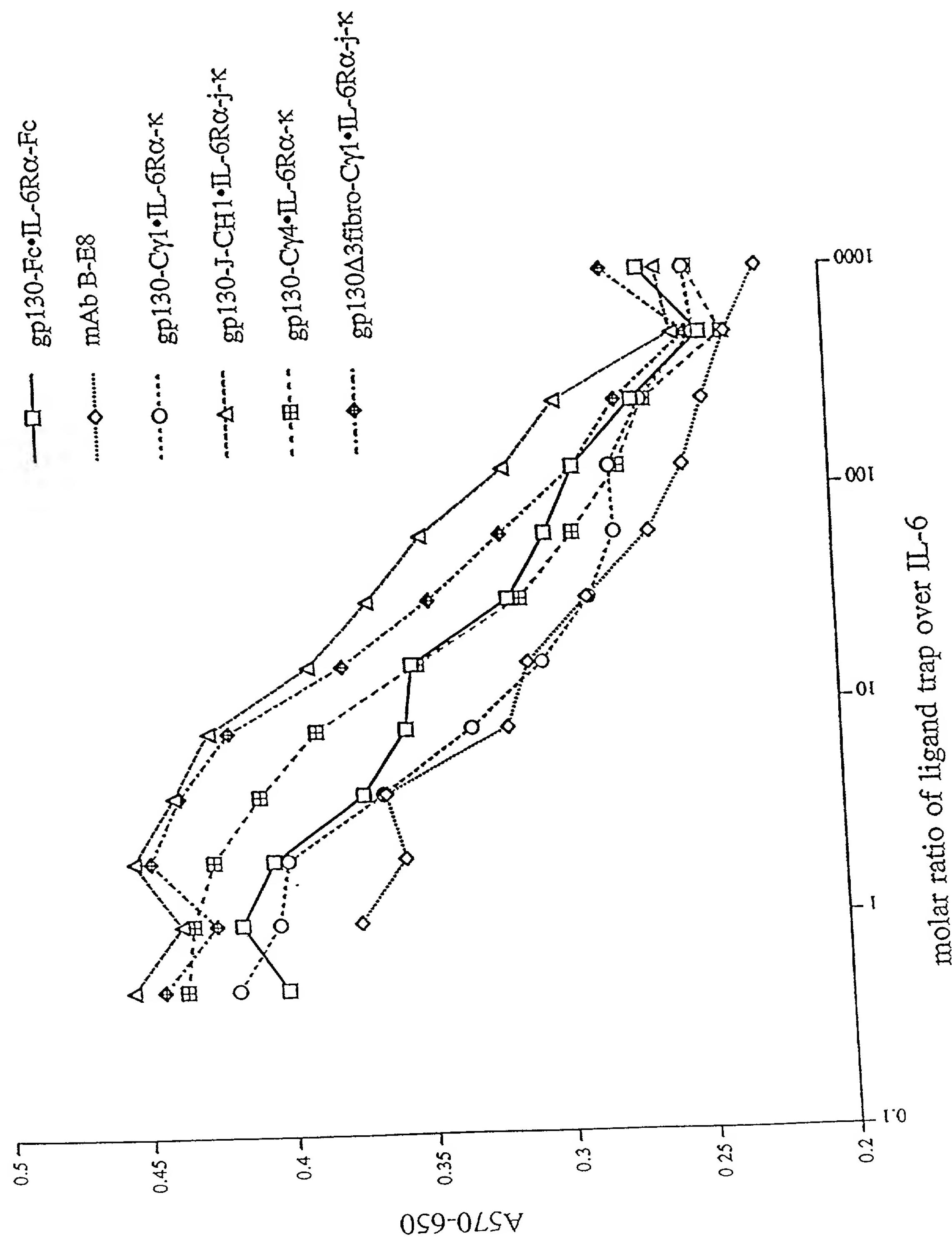


IP: Prot A

Blot: α -kappa

FIGURE. 20

XG-1 cell proliferation assay



	10	20	30	40												
*	*	*	*	*	*											
ATG	GTG	AAG	CCA	TCA	TTA	CCA	TTC	ACA	TCC	CTC	TTA	TTC	CTG	CAG	CTG	
Met	Val	Lys	Pro	Ser	Leu	Pro	Phe	Thr	Ser	Leu	Leu	Phe	Leu	Gln	Leu	>
	50	60	70	80	90											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CCC	CTG	CTG	GGA	GTG	GGG	CTG	AAC	ACG	ACA	ATT	CTG	ACG	CCC	AAT	GGG	
Pro	Leu	Leu	Gly	Val	Gly	Leu	Asn	Thr	Thr	Ile	Leu	Thr	Pro	Asn	Gly	>
	100	110	120	130	140											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
AAT	GAA	GAC	ACC	ACA	GCT	GAT	TTC	TTC	CTG	ACC	ACT	ATG	CCC	ACT	GAC	
Asn	Glu	Asp	Thr	Thr	Ala	Asp	Phe	Phe	Leu	Thr	Thr	Met	Pro	Thr	Asp	>
	150	160	170	180	190											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
TCC	CTC	AGT	GTT	TCC	ACT	CTG	CCC	CTC	CCA	GAG	GTT	CAG	TGT	TTT	GTG	
Ser	Leu	Ser	Val	Ser	Thr	Leu	Pro	Leu	Pro	Glu	Val	Gln	Cys	Phe	Val	>
	200	210	220	230	240											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
TTC	AAT	GTC	GAG	TAC	ATG	AAT	TGC	ACT	TGG	AAC	AGC	AGC	TCT	GAG	CCC	
Phe	Asn	Val	Glu	Tyr	Met	Asn	Cys	Thr	Trp	Asn	Ser	Ser	Ser	Glu	Pro	>
	250	260	270	280												
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CAG	CCT	ACC	AAC	CTC	ACT	CTG	CAT	TAT	TGG	TAC	AAG	AAC	TCG	GAT	AAT	
Gln	Pro	Thr	Asn	Leu	Thr	Leu	His	Tyr	Trp	Tyr	Lys	Asn	Ser	Asp	Asn	>
	290	300	310	320	330											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
GAT	AAA	GTC	CAG	AAG	TGC	AGC	CAC	TAT	CTA	TTC	TCT	GAA	GAA	ATC	ACT	
Asp	Lys	Val	Gln	Lys	Cys	Ser	His	Tyr	Leu	Phe	Ser	Glu	Glu	Ile	Thr	>
	340	350	360	370	380											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
TCT	GGC	TGT	CAG	TTG	CAA	AAA	AAG	GAG	ATC	CAC	CTC	TAC	CAA	ACA	TTT	
Ser	Gly	Cys	Gln	Leu	Gln	Lys	Lys	Glu	Ile	His	Leu	Tyr	Gln	Thr	Phe	>
	390	400	410	420	430											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
GTT	GTT	CAG	CTC	CAG	GAC	CCA	CGG	GAA	CCC	AGG	AGA	CAG	GCC	ACA	CAG	
Val	Val	Gln	Leu	Gln	Asp	Pro	Arg	Glu	Pro	Arg	Arg	Gln	Ala	Thr	Gln	>
	440	450	460	470	480											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
ATG	CTA	AAA	CTG	CAG	AAT	CTG	GTG	ATC	CCC	TGG	GCT	CCA	GAG	AAC	CTA	
Met	Leu	Lys	Leu	Gln	Asn	Leu	Val	Ile	Pro	Trp	Ala	Pro	Glu	Asn	Leu	>
	490	500	510	520												
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
ACA	CTT	CAC	AAA	CTG	AGT	GAA	TCC	CAG	CTA	GAA	CTG	AAC	TGG	AAC	AAC	
Thr	Leu	His	Lys	Leu	Ser	Glu	Ser	Gln	Leu	Glu	Leu	Asn	Trp	Asn	Asn	>
	530	540	550	560	570											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
AGA	TTC	TTG	AAC	CAC	TGT	TTG	GAG	CAC	TTG	GTG	CAG	TAC	CGG	ACT	GAC	
Arg	Phe	Leu	Asn	His	Cys	Leu	Glu	His	Leu	Val	Gln	Tyr	Arg	Thr	Asp	>

Figure 21B

580 590 600 610 620
 * * * * *
 TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC
 Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe>

630 640 650 660 670
 * * * * *
 TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG
 Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg>

680 690 700 710 720
 * * * * *
 AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG
 Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp>

730 740 750 760
 * * * * *
 AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GCG TCG
 Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Ala Ser>

770 780 790 800 810
 * * * * *
 TCT GGG AAC ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC
 Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr>

820 830 840 850 860
 * * * * *
 ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC
 Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys>

870 880 890 900 910
 * * * * *
 AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA
 Ser Thr Glu Leu Arg Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu>

920 930 940 950 960
 * * * * *
 GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC
 Ala His Thr Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys>

970 980 990 1000
 * * * * *
 CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC
 His Leu Leu Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp>

1010 1020 1030 1040 1050
 * * * * *
 CTG TGG GCT GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC
 Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser>

1060 1070 1080 1090 1100
 * * * * *
 GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT
 Glu His Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn>

1110 1120 1130 1140 1150
 * * * * *
 GTC TCC GAC ACT CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC
 Val Ser Asp Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp>

1160 1170 1180 1190 1200
 * * * * *

Figure 21C

AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA
Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu>

	1210	1220	1230	1240	
*	*	*	*	*	*

AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC
Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro>

1250	1260	1270	1280	1290	
*	*	*	*	*	*

TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG
Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg>

1300	1310	1320	1330	1340	
*	*	*	*	*	*

GCA CGG GTG AGG GCC TGG GCT CAG TGC TAT AAC ACC ACC TGG AGT GAG
Ala Arg Val Arg Ala Trp Ala Gln Cys Tyr Asn Thr Trp Ser Glu>

1350	1360	1370	1380	1390	
*	*	*	*	*	*

TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG
Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu>

1400	1410	1420	1430	1440	
*	*	*	*	*	*

CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA
Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu>

1450	1460	1470	1480		
*	*	*	*	*	*

CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp>

1490	1500	1510	1520	1530	
*	*	*	*	*	*

ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp>

1540	1550	1560	1570	1580	
*	*	*	*	*	*

GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly>

1590	1600	1610	1620	1630	
*	*	*	*	*	*

GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG CAG TAC AAC
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn>

1640	1650	1660	1670	1680	
*	*	*	*	*	*

AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp>

1690	1700	1710	1720		
*	*	*	*	*	*

CTG AAT GCC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro>

1730	1740	1750	1760	1770	
*	*	*	*	*	*

GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

Figure 21D

1780	1790	1800	1810	1820
*	*	*	*	*
CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC				
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn>				
1830	1840	1850	1860	1870
*	*	*	*	*
CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC				
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile>				
1880	1890	1900	1910	1920
*	*	*	*	*
GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC				
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr>				
1930	1940	1950	1960	
*	*	*	*	*
ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG				
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys>				
1970	1980	1990	2000	2010
*	*	*	*	*
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC				
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys>				
2020	2030	2040	2050	2060
*	*	*	*	*
TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC				
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu>				
2070	2080			
*	*	*	*	*
TCC CTG TCT CCG GGT AAA TGA				
Ser Leu Ser Pro Gly Lys ***>				

Figure 22A

	10	20	30	40	
*	*	*	*	*	*
ATG GTG AAG CCA TCA TTA CCA TTC ACA TCC CTC TTA TTC CTG CAG CTG					
Met Val Lys Pro Ser Leu Pro Phe Thr Ser Leu Leu Phe Leu Gln Leu>					
	50	60	70	80	90
*	*	*	*	*	*
CCC CTG CTG GGA GTG GGG CTG AAC ACG ACA ATT CTG ACG CCC AAT GGG					
Pro Leu Leu Gly Val Gly Leu Asn Thr Thr Ile Leu Thr Pro Asn Gly>					
	100	110	120	130	140
*	*	*	*	*	*
AAT GAA GAC ACC ACA GCT GAT TTC TTC CTG ACC ACT ATG CCC ACT GAC					
Asn Glu Asp Thr Thr Ala Asp Phe Phe Leu Thr Thr Met Pro Thr Asp>					
	150	160	170	180	190
*	*	*	*	*	*
TCC CTC AGT GTT TCC ACT CTG CCC CTC CCA GAG GTT CAG TGT TTT GTG					
Ser Leu Ser Val Ser Thr Leu Pro Leu Pro Glu Val Gln Cys Phe Val>					
	200	210	220	230	240
*	*	*	*	*	*
TTC AAT GTC GAG TAC ATG AAT TGC ACT TGG AAC AGC AGC TCT GAG CCC					
Phe Asn Val Glu Tyr Met Asn Cys Thr Trp Asn Ser Ser Glu Pro>					
	250	260	270	280	
*	*	*	*	*	*
CAG CCT ACC AAC CTC ACT CTG CAT TAT TGG TAC AAG AAC TCG GAT AAT					
Gln Pro Thr Asn Leu Thr Leu His Tyr Trp Tyr Lys Asn Ser Asp Asn>					
	290	300	310	320	330
*	*	*	*	*	*
GAT AAA GTC CAG AAG TGC AGC CAC TAT CTA TTC TCT GAA GAA ATC ACT					
Asp Lys Val Gln Lys Cys Ser His Tyr Leu Phe Ser Glu Glu Ile Thr>					
	340	350	360	370	380
*	*	*	*	*	*
TCT GGC TGT CAG TTG CAA AAA AAG GAG ATC CAC CTC TAC CAA ACA TTT					
Ser Gly Cys Gln Leu Gln Lys Lys Glu Ile His Leu Tyr Gln Thr Phe>					
	390	400	410	420	430
*	*	*	*	*	*
GTT GTT CAG CTC CAG GAC CCA CGG GAA CCC AGG AGA CAG GCC ACA CAG					
Val Val Gln Leu Gln Asp Pro Arg Glu Pro Arg Arg Gln Ala Thr Gln>					
	440	450	460	470	480
*	*	*	*	*	*
ATG CTA AAA CTG CAG AAT CTG GTG ATC CCC TGG GCT CCA GAG AAC CTA					
Met Leu Lys Leu Gln Asn Leu Val Ile Pro Trp Ala Pro Glu Asn Leu>					
	490	500	510	520	
*	*	*	*	*	*
ACA CTT CAC AAA CTG AGT GAA TCC CAG CTA GAA CTG AAC TGG AAC AAC					
Thr Leu His Lys Leu Ser Glu Ser Gln Leu Glu Leu Asn Trp Asn Asn>					
	530	540	550	560	570
*	*	*	*	*	*
AGA TTC TTG AAC CAC TGT TTG GAG CAC TTG GTG CAG TAC CGG ACT GAC					
Arg Phe Leu Asn His Cys Leu Glu His Leu Val Gln Tyr Arg Thr Asp>					

Figure 22B

580	590	600	610	620
*	*	*	*	*
TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe>				
630	640	650	660	670
*	*	*	*	*
TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg>				
680	690	700	710	720
*	*	*	*	*
AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp>				
730	740	750	760	
*	*	*	*	*
AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GGG AAC Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Gly Asn>				
770	780	790	800	810
*	*	*	*	*
ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC ATG AGC ATC Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr Met Ser Ile>				
820	830	840	850	860
*	*	*	*	*
TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC AGC ACC GAG Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys Ser Thr Glu>				
870	880	890	900	910
*	*	*	*	*
CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA GCC CAC ACG Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu Ala His Thr>				
920	930	940	950	960
*	*	*	*	*
TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC CAC CTG CTC Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys His Leu Leu>				
970	980	990	1000	
*	*	*	*	*
ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC CTG TGG GCT Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp Leu Trp Ala>				
1010	1020	1030	1040	1050
*	*	*	*	*
GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC GAG CAT GTG Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser Glu His Val>				
1060	1070	1080	1090	1100
*	*	*	*	*
AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT GTC TCC GAC Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn Val Ser Asp>				
1110	1120	1130	1140	1150
*	*	*	*	*
ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC AAT TAC CTG Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp Asn Tyr Leu>				
1160	1170	1180	1190	1200
*	*	*	*	*

Figure 22C

TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA AAC GAC CCG
 Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu Asn Asp Pro>

	1210	1220	1230	1240	
*	*	*	*	*	*
GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC TCC CTC CGC					
Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro Ser Leu Arg>					
	1250	1260	1270	1280	1290
*	*	*	*	*	*
ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG GCA CGG GTG					
Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg Ala Arg Val>					
	1300	1310	1320	1330	1340
*	*	*	*	*	*
AGG GCC TGG GCT CAG AGC TAT AAC ACC ACC TGG AGT GAG TGG AGC CCC					
Arg Ala Trp Ala Gln Ser Tyr Asn Thr Thr Trp Ser Glu Trp Ser Pro>					
	1350	1360	1370	1380	1390
*	*	*	*	*	*
AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG CAG TCC GGA					
Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu Gln Ser Gly>					
	1400	1410	1420	1430	1440
*	*	*	*	*	*
GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG					
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly>					
	1450	1460	1470	1480	
*	*	*	*	*	*
GGA CCG TCA GTC TTC CTC CCC CCA AAA CCC AAG GAC ACC CTC ATG					
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met>					
	1490	1500	1510	1520	1530
*	*	*	*	*	*
ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC GTG AGC CAC					
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His>					
	1540	1550	1560	1570	1580
*	*	*	*	*	*
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG					
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val>					
	1590	1600	1610	1620	1630
*	*	*	*	*	*
CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC					
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr>					
	1640	1650	1660	1670	1680
*	*	*	*	*	*
CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC					
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly>					
	1690	1700	1710	1720	
*	*	*	*	*	*
AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC					
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile>					
	1730	1740	1750	1760	1770
*	*	*	*	*	*
GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG					
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val>					

Figure 22D

1780	1790	1800	1810	1820
*	*	*	*	*
TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser>				
1830	1840	1850	1860	1870
*	*	*	*	*
CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu>				
1880	1890	1900	1910	1920
*	*	*	*	*
TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro>				
1930	1940	1950	1960	
*	*	*	*	*
GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG CTC ACC GTG Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val>				
1970	1980	1990	2000	2010
*	*	*	*	*
GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met>				
2020	2030	2040	2050	2060
*	*	*	*	*
CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser>				
2070				
*	*	*		
CCG GGT AAA TGA Pro Gly Lys ***>				

Figure 23A

	10	20	30	40												
*	*	*	*	*	*											
ATG	GTG	AAG	CCA	TCA	TTA	CCA	TTC	ACA	TCC	CTC	TTA	TTC	CTG	CAG	CTG	
Met	Val	Lys	Pro	Ser	Leu	Pro	Phe	Thr	Ser	Leu	Leu	Phe	Leu	Gln	Leu	>
	50	60	70	80	90											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CCC	CTG	CTG	GGA	GTG	GGG	CTG	AAC	ACG	ACA	ATT	CTG	ACG	CCC	AAT	GGG	
Pro	Leu	Leu	Gly	Val	Gly	Leu	Asn	Thr	Thr	Ile	Leu	Thr	Pro	Asn	Gly	>
	100	110	120	130	140											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
AAT	GAA	GAC	ACC	ACA	GCT	GAT	TTC	TTC	CTG	ACC	ACT	ATG	CCC	ACT	GAC	
Asn	Glu	Asp	Thr	Thr	Ala	Asp	Phe	Phe	Leu	Thr	Thr	Met	Pro	Thr	Asp	>
	150	160	170	180	190											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
TCC	CTC	AGT	GTT	TCC	ACT	CTG	CCC	CTC	CCA	GAG	GTT	CAG	TGT	TTT	GTG	
Ser	Leu	Ser	Val	Ser	Thr	Leu	Pro	Leu	Pro	Glu	Val	Gln	Cys	Phe	Val	>
	200	210	220	230	240											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
TTC	AAT	GTC	GAG	TAC	ATG	AAT	TGC	ACT	TGG	AAC	AGC	AGC	TCT	GAG	CCC	
Phe	Asn	Val	Glu	Tyr	Met	Asn	Cys	Thr	Trp	Asn	Ser	Ser	Ser	Glu	Pro	>
	250	260	270	280												
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CAG	CCT	ACC	AAC	CTC	ACT	CTG	CAT	TAT	TGG	TAC	AAG	AAC	TCG	GAT	AAT	
Gln	Pro	Thr	Asn	Leu	Thr	Leu	His	Tyr	Trp	Tyr	Lys	Asn	Ser	Asp	Asn	>
	290	300	310	320	330											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
GAT	AAA	GTC	CAG	AAG	TGC	AGC	CAC	TAT	CTA	TTC	TCT	GAA	GAA	ATC	ACT	
Asp	Lys	Val	Gln	Lys	Cys	Ser	His	Tyr	Leu	Phe	Ser	Glu	Glu	Ile	Thr	>
	340	350	360	370	380											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
TCT	GGC	TGT	CAG	TTG	CAA	AAA	AAG	GAG	ATC	CAC	CTC	TAC	CAA	ACA	TTT	
Ser	Gly	Cys	Gln	Leu	Gln	Lys	Lys	Glu	Ile	His	Leu	Tyr	Gln	Thr	Phe	>
	390	400	410	420	430											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
GTT	GTT	CAG	CTC	CAG	GAC	CCA	CGG	GAA	CCC	AGG	AGA	CAG	GCC	ACA	CAG	
Val	Val	Gln	Leu	Gln	Asp	Pro	Arg	Glu	Pro	Arg	Arg	Gln	Ala	Thr	Gln	>
	440	450	460	470	480											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
ATG	CTA	AAA	CTG	CAG	AAT	CTG	GTG	ATC	CCC	TGG	GCT	CCA	GAG	AAC	CTA	
Met	Leu	Lys	Leu	Gln	Asn	Leu	Val	Ile	Pro	Trp	Ala	Pro	Glu	Asn	Leu	>
	490	500	510	520												
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
ACA	CTT	CAC	AAA	CTG	AGT	GAA	TCC	CAG	CTA	GAA	CTG	AAC	TGG	AAC	AAC	
Thr	Leu	His	Lys	Leu	Ser	Glu	Ser	Gln	Leu	Glu	Leu	Asn	Trp	Asn	Asn	>
	530	540	550	560	570											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
AGA	TTC	TTG	AAC	CAC	TGT	TTG	GAG	CAC	TTG	GTG	CAG	TAC	CGG	ACT	GAC	
Arg	Phe	Leu	Asn	His	Cys	Leu	Glu	His	Leu	Val	Gln	Tyr	Arg	Thr	Asp	>

32/69
Figure 23B

580	590	600	610	620
*	*	*	*	*
TGG GAC CAC AGC TGG ACT GAA CAA TCA GTG GAT TAT AGA CAT AAG TTC				
Trp Asp His Ser Trp Thr Glu Gln Ser Val Asp Tyr Arg His Lys Phe>				
630	640	650	660	670
*	*	*	*	*
TCC TTG CCT AGT GTG GAT GGG CAG AAA CGC TAC ACG TTT CGT GTT CGG				
Ser Leu Pro Ser Val Asp Gly Gln Lys Arg Tyr Thr Phe Arg Val Arg>				
680	690	700	710	720
*	*	*	*	*
AGC CGC TTT AAC CCA CTC TGT GGA AGT GCT CAG CAT TGG AGT GAA TGG				
Ser Arg Phe Asn Pro Leu Cys Gly Ser Ala Gln His Trp Ser Glu Trp>				
730	740	750	760	
*	*	*	*	
AGC CAC CCA ATC CAC TGG GGG AGC AAT ACT TCA AAA GAG AAC GCG TCG				
Ser His Pro Ile His Trp Gly Ser Asn Thr Ser Lys Glu Asn Ala Ser>				
770	780	790	800	810
*	*	*	*	*
TCT GGG AAC ATG AAG GTC CTG CAG GAG CCC ACC TGC GTC TCC GAC TAC				
Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val Ser Asp Tyr>				
820	830	840	850	860
*	*	*	*	*
ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC ACC AAT TGC				
Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro Thr Asn Cys>				
870	880	890	900	910
*	*	*	*	*
AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG CTC TCC GAA				
Ser Thr Glu Leu Arg Leu Tyr Gln Leu Val Phe Leu Leu Ser Glu>				
920	930	940	950	960
*	*	*	*	*
GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG TGC GTG TGC				
Ala His Thr Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly Cys Val Cys>				
970	980	990	1000	
*	*	*	*	
CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT ACA CTG GAC				
His Leu Leu Met Asp Asp Val Val Ser Ala Asp Asn Tyr Thr Leu Asp>				
1010	1020	1030	1040	1050
*	*	*	*	*
CTG TGG GCT GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC AAG CCC AGC				
Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe Lys Pro Ser>				
1060	1070	1080	1090	1100
*	*	*	*	*
GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT CAC ACC AAT				
Glu His Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val His Thr Asn>				
1110	1120	1130	1140	1150
*	*	*	*	*
GTC TCC GAC ACT CTG CTG ACC TGG AGC AAC CCG TAT CCC CCT GAC				
Val Ser Asp Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr Pro Pro Asp>				
1160	1170	1180	1190	1200
*	*	*	*	*

AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT TGG AGT GAA
Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile Trp Ser Glu>

1210	1220	1230	1240	
*	*	*	*	*

AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC CTA GAA CCC
Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr Leu Glu Pro>

1250	1260	1270	1280	1290	
*	*	*	*	*	*

TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT TCC TAC AGG
Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile Ser Tyr Arg>

1300	1310	1320	1330	1340	
*	*	*	*	*	*

GCA CGG GTG AGG GCC TGG GCT CAG AGC TAT AAC ACC ACC TGG AGT GAG
Ala Arg Val Arg Ala Trp Ala Gln Ser Tyr Asn Thr Thr Trp Ser Glu>

1350	1360	1370	1380	1390	
*	*	*	*	*	*

TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG CCC TTC GAG
Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu Pro Phe Glu>

1400	1410	1420	1430	1440	
*	*	*	*	*	*

CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA
Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu>

1450	1460	1470	1480		
*	*	*	*	*	*

CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp>

1490	1500	1510	1520	1530	
*	*	*	*	*	*

ACC CTC ATG ATC TCC CCG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp>

1540	1550	1560	1570	1580	
*	*	*	*	*	*

GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly>

1590	1600	1610	1620	1630	
*	*	*	*	*	*

GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG CAG TAC AAC
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn>

1640	1650	1660	1670	1680	
*	*	*	*	*	*

AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp>

1690	1700	1710	1720		
*	*	*	*	*	*

CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro>

1730	1740	1750	1760	1770	
*	*	*	*	*	*

GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>

Figure 23D

1780	1790	1800	1810	1820
*	*	*	*	*
CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC				
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn>				
1830	1840	1850	1860	1870
*	*	*	*	*
CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC				
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile>				
1880	1890	1900	1910	1920
*	*	*	*	*
GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC				
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr>				
1930	1940	1950	1960	
*	*	*	*	*
ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG				
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys>				
1970	1980	1990	2000	2010
*	*	*	*	*
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC				
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys>				
2020	2030	2040	2050	2060
*	*	*	*	*
TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC				
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu>				
2070	2080			
*	*	*	*	*
TCC CTG TCT CCG GGT AAA TGA				
Ser Leu Ser Pro Gly Lys ***>				

Figure 24A

10	20	30	40	
*	*	*	*	
ATG GTG GCC GTC GGC TGC GCG CTG CTG GCT GCC CTG CTG GCC GCG CCG				
Met Val Ala Val Gly Cys Ala Leu Leu Ala Ala Leu Leu Ala Ala Pro>				
50	60	70	80	90
*	*	*	*	*
GGA GCG GCG CTG GCC CCA AGG CGC TGC CCT GCG CAG GAG GTG GCA AGA				
Gly Ala Ala Leu Ala Pro Arg Arg Cys Pro Ala Gln Glu Val Ala Arg>				
100	110	120	130	140
*	*	*	*	*
GGC GTG CTG ACC AGT CTG CCA GGA GAC AGC GTG ACT CTG ACC TGC CCG				
Gly Val Leu Thr Ser Leu Pro Gly Asp Ser Val Thr Leu Thr Cys Pro>				
150	160	170	180	190
*	*	*	*	*
GGG GTA GAG CCG GAA GAC AAT GCC ACT GTT CAC TGG GTG CTC AGG AAG				
Gly Val Glu Pro Glu Asp Asn Ala Thr Val His Trp Val Leu Arg Lys>				
200	210	220	230	240
*	*	*	*	*
CCG GCT GCA GGC TCC CAC CCC AGC AGA TGG GCT GGC ATG GGA AGG AGG				
Pro Ala Ala Gly Ser His Pro Ser Arg Trp Ala Gly Met Gly Arg Arg>				
250	260	270	280	
*	*	*	*	
CTG CTG CTG AGG TCG GTG CAG CTC CAC GAC TCT GGA AAC TAT TCA TGC				
Leu Leu Leu Arg Ser Val Gln Leu His Asp Ser Gly Asn Tyr Ser Cys>				
290	300	310	320	330
*	*	*	*	*
TAC CGG GCC GGC CGC CCA GCT GGG ACT GTG CAC TTG CTG GTG GAT GTT				
Tyr Arg Ala Gly Arg Pro Ala Gly Thr Val His Leu Leu Val Asp Val>				
340	350	360	370	380
*	*	*	*	*
CCC CCC GAG GAG CCC CAG CTC TCC TGC TTC CGG AAG AGC CCC CTC AGC				
Pro Pro Glu Glu Pro Gln Leu Ser Cys Phe Arg Lys Ser Pro Leu Ser>				
390	400	410	420	430
*	*	*	*	*
AAT GTT GTT TGT GAG TGG GGT CCT CGG AGC ACC CCA TCC CTG ACG ACA				
Asn Val Val Cys Glu Trp Gly Pro Arg Ser Thr Pro Ser Leu Thr Thr>				
440	450	460	470	480
*	*	*	*	*
AAG GCT GTG CTC TTG GTG AGG AAG TTT CAG AAC AGT CCG GCC GAA GAC				
Lys Ala Val Leu Leu Val Arg Lys Phe Gln Asn Ser Pro Ala Glu Asp>				
490	500	510	520	
*	*	*	*	
TTC CAG GAG CCG TGC CAG TAT TCC CAG GAG TCC CAG AAG TTC TCC TGC				
Phe Gln Glu Pro Cys Gln Tyr Ser Gln Glu Ser Gln Lys Phe Ser Cys>				
530	540	550	560	570
*	*	*	*	*
CAG TTA GCA GTC CCG GAG GGA GAC AGC TCT TTC TAC ATA GTG TCC ATG				
Gln Leu Ala Val Pro Glu Gly Asp Ser Ser Phe Tyr Ile Val Ser Met>				

Figure 24B

580	590	600	610	620
*	*	*	*	*
TGC GTC GCC AGT AGT GTC GGG AGC AAG TTC AGC AAA ACT CAA ACC TTT Cys Val Ala Ser Ser Val Gly Ser Lys Phe Ser Lys Thr Gln Thr Phe>				
630	640	650	660	670
*	*	*	*	*
CAG GGT TGT GGA ATC TTG CAG CCT GAT CCG CCT GCC AAC ATC ACA GTC Gln Gly Cys Gly Ile Leu Gln Pro Asp Pro Pro Ala Asn Ile Thr Val>				
680	690	700	710	720
*	*	*	*	*
ACT GCC GTG GCC AGA AAC CCC CGC TGG CTC AGT GTC ACC TGG CAA GAC Thr Ala Val Ala Arg Asn Pro Arg Trp Leu Ser Val Thr Trp Gln Asp>				
730	740	750	760	
*	*	*	*	*
CCC CAC TCC TGG AAC TCA TCT TTC TAC AGA CTA CGG TTT GAG CTC AGA Pro His Ser Trp Asn Ser Ser Phe Tyr Arg Leu Arg Phe Glu Leu Arg>				
770	780	790	800	810
*	*	*	*	*
TAT CGG GCT GAA CGG TCA AAG ACA TTC ACA ACA TGG ATG GTC AAG GAC Tyr Arg Ala Glu Arg Ser Lys Thr Phe Thr Thr Trp Met Val Lys Asp>				
820	830	840	850	860
*	*	*	*	*
CTC CAG CAT CAC TGT GTC ATC CAC GAC GCC TGG AGC GGC CTG AGG CAC Leu Gln His His Cys Val Ile His Asp Ala Trp Ser Gly Leu Arg His>				
870	880	890	900	910
*	*	*	*	*
GTG GTG CAG CTT CGT GCC CAG GAG TTC GGG CAA GGC GAG TGG AGC Val Val Gln Leu Arg Ala Gln Glu Phe Gly Gln Gly Glu Trp Ser>				
920	930	940	950	960
*	*	*	*	*
GAG TGG AGC CCG GAG GCC ATG GGC ACG CCT TGG ACA GAA TCC AGG AGT Glu Trp Ser Pro Glu Ala Met Gly Thr Pro Trp Thr Glu Ser Arg Ser>				
970	980	990	1000	
*	*	*	*	*
CCT CCA GCT GAG AAC GAG GTG TCC ACC CCC ATG ACC GGT GGC GCG CCT Pro Pro Ala Glu Asn Glu Val Ser Thr Pro Met Thr Gly Gly Ala Pro>				
1010	1020	1030	1040	1050
*	*	*	*	*
TCA GGT GCT CAG CTG GAA CTT CTA GAC CCA TGT GGT TAT ATC AGT CCT Ser Gly Ala Gln Leu Glu Leu Leu Asp Pro Cys Gly Tyr Ile Ser Pro>				
1060	1070	1080	1090	1100
*	*	*	*	*
GAA TCT CCA GTT GTA CAA CTT CAT TCT AAT TTC ACT GCA GTT TGT GTG Glu Ser Pro Val Val Gln Leu His Ser Asn Phe Thr Ala Val Cys Val>				
1110	1120	1130	1140	1150
*	*	*	*	*
CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT GTA AAT GCT AAT TAC ATT Leu Lys Glu Lys Cys Met Asp Tyr Phe His Val Asn Ala Asn Tyr Ile>				
1160	1170	1180	1190	1200
*	*	*	*	*

Figure 24C

GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT AAG GAG CAA TAT ACT ATC
 Val Trp Lys Thr Asn His Phe Thr Ile Pro Lys Glu Gln Tyr Thr Ile>

1210	1220	1230	1240
*	*	*	*

ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT ACA GAT ATA GCT TCA TTA
 Ile Asn Arg Thr Ala Ser Ser Val Thr Phe Thr Asp Ile Ala Ser Leu>

1250	1260	1270	1280	1290
*	*	*	*	*

AAT ATT CAG CTC ACT TGC AAC ATT CTT ACA TTC GGA CAG CTT GAA CAG
 Asn Ile Gln Leu Thr Cys Asn Ile Leu Thr Phe Gly Gln Leu Glu Gln>

1300	1310	1320	1330	1340
*	*	*	*	*

AAT GTT TAT GGA ATC ACA ATA ATT TCA GGC TTG CCT CCA GAA AAA CCT
 Asn Val Tyr Gly Ile Thr Ile Ser Gly Leu Pro Pro Glu Lys Pro>

1350	1360	1370	1380	1390
*	*	*	*	*

AAA AAT TTG AGT TGC ATT GTG AAC GAG GGG AAG AAA ATG AGG TGT GAG
 Lys Asn Leu Ser Cys Ile Val Asn Glu Gly Lys Lys Met Arg Cys Glu>

1400	1410	1420	1430	1440
*	*	*	*	*

TGG GAT GGT GGA AGG GAA ACA CAC TTG GAG ACA AAC TTC ACT TTA AAA
 Trp Asp Gly Gly Arg Glu Thr His Leu Glu Thr Asn Phe Thr Leu Lys>

1450	1460	1470	1480
*	*	*	*

TCT GAA TGG GCA ACA CAC AAG TTT GCT GAT TGC AAA GCA AAA CGT GAC
 Ser Glu Trp Ala Thr His Lys Phe Ala Asp Cys Lys Ala Lys Arg Asp>

1490	1500	1510	1520	1530
*	*	*	*	*

ACC CCC ACC TCA TGC ACT GTT GAT TAT TCT ACT GTG TAT TTT GTC AAC
 Thr Pro Thr Ser Cys Thr Val Asp Tyr Ser Thr Val Tyr Phe Val Asn>

1540	1550	1560	1570	1580
*	*	*	*	*

ATT GAA GTC TGG GTA GAA GCA GAG AAT GCC CTT GGG AAG GTT ACA TCA
 Ile Glu Val Trp Val Glu Ala Glu Asn Ala Leu Gly Lys Val Thr Ser>

1590	1600	1610	1620	1630
*	*	*	*	*

GAT CAT ATC AAT TTT GAT CCT GTA TAT AAA GTG AAG CCC AAT CCG CCA
 Asp His Ile Asn Phe Asp Pro Val Tyr Lys Val Lys Pro Asn Pro Pro>

1640	1650	1660	1670	1680
*	*	*	*	*

CAT AAT TTA TCA GTG ATC AAC TCA GAG GAA CTG TCT AGT ATC TTA AAA
 His Asn Leu Ser Val Ile Asn Ser Glu Glu Leu Ser Ser Ile Leu Lys>

1690	1700	1710	1720
*	*	*	*

TTG ACA TGG ACC AAC CCA AGT ATT AAG AGT GTT ATA ATA CTA AAA TAT
 Leu Thr Trp Thr Asn Pro Ser Ile Lys Ser Val Ile Ile Leu Lys Tyr>

1730	1740	1750	1760	1770
*	*	*	*	*

AAC ATT CAA TAT AGG ACC AAA GAT GCC TCA ACT TGG AGC CAG ATT CCT
 Asn Ile Gln Tyr Arg Thr Lys Asp Ala Ser Thr Trp Ser Gln Ile Pro>

Figure 24D

1780	1790	1800	1810	1820											
*	*	*	*	*											
CCT	GAA	GAC	ACA	GCA	TCC	ACC	CGA	TCT	TCA	TTC	ACT	GTC	CAA	GAC	CTT
Pro	Glu	Asp	Thr	Ala	Ser	Thr	Arg	Ser	Ser	Phe	Thr	Val	Gln	Asp	Leu
1830	1840	1850	1860	1870											
*	*	*	*	*											
AAA	CCT	TTT	ACA	GAA	TAT	GTG	TTT	AGG	ATT	CGC	TGT	ATG	AAG	GAA	GAT
Lys	Pro	Phe	Thr	Glu	Tyr	Val	Phe	Arg	Ile	Arg	Cys	Met	Lys	Glu	Asp
1880	1890	1900	1910	1920											
*	*	*	*	*											
GGT	AAG	GGA	TAC	TGG	AGT	GAC	TGG	AGT	GAA	GAA	GCA	AGT	GGG	ATC	ACC
Gly	Lys	Gly	Tyr	Trp	Ser	Asp	Trp	Ser	Glu	Glu	Ala	Ser	Gly	Ile	Thr
1930	1940	1950	1960												
*	*	*	*	*											
TAT	GAA	GAT	AGA	CCA	TCT	AAA	GCA	CCA	AGT	TTC	TGG	TAT	AAA	ATA	GAT
Tyr	Glu	Asp	Arg	Pro	Ser	Lys	Ala	Pro	Ser	Phe	Trp	Tyr	Lys	Ile	Asp
1970	1980	1990	2000	2010											
*	*	*	*	*											
CCA	TCC	CAT	ACT	CAA	GGC	TAC	AGA	ACT	GTA	CAA	CTC	GTG	TGG	AAG	ACA
Pro	Ser	His	Thr	Gln	Gly	Tyr	Arg	Thr	Val	Gln	Leu	Val	Trp	Lys	Thr
2020	2030	2040	2050	2060											
*	*	*	*	*											
TTG	CCT	TTT	GAA	GCC	AAT	GGA	AAA	ATC	TTG	GAT	TAT	GAA	GTG	ACT	
Leu	Pro	Pro	Phe	Glu	Ala	Asn	Gly	Ile	Leu	Asp	Tyr	Glu	Val	Thr	
2070	2080	2090	2100	2110											
*	*	*	*	*											
CTC	ACA	AGA	TGG	AAA	TCA	CAT	TTA	CAA	AAT	TAC	ACA	GTT	AAT	GCC	ACA
Leu	Thr	Arg	Trp	Lys	Ser	His	Leu	Gln	Asn	Tyr	Thr	Val	Asn	Ala	Thr
2120	2130	2140	2150	2160											
*	*	*	*	*											
AAA	CTG	ACA	GTA	AAT	CTC	ACA	AAT	GAT	CGC	TAT	CTA	GCA	ACC	CTA	ACA
Lys	Leu	Thr	Val	Asn	Leu	Thr	Asn	Asp	Arg	Tyr	Leu	Ala	Thr	Leu	Thr
2170	2180	2190	2200												
*	*	*	*	*											
GTA	AGA	AAT	CTT	GTT	GGC	AAA	TCA	GAT	GCA	GCT	GTT	TTA	ACT	ATC	CCT
Val	Arg	Asn	Leu	Val	Gly	Lys	Ser	Asp	Ala	Ala	Val	Leu	Thr	Ile	Pro
2210	2220	2230	2240	2250											
*	*	*	*	*											
GCC	TGT	GAC	TTT	CAA	GCT	ACT	CAC	CCT	GTA	ATG	GAT	CTT	AAA	GCA	TTC
Ala	Cys	Asp	Phe	Gln	Ala	Thr	His	Pro	Val	Met	Asp	Leu	Lys	Ala	Phe
2260	2270	2280	2290	2300											
*	*	*	*	*											
CCC	AAA	GAT	AAC	ATG	CTT	TGG	GTG	GAA	TGG	ACT	ACT	CCA	AGG	GAA	TCT
Pro	Lys	Asp	Asn	Met	Leu	Trp	Val	Glu	Trp	Thr	Thr	Pro	Arg	Glu	Ser
2310	2320	2330	2340	2350											
*	*	*	*	*											
GTA	AAG	AAA	TAT	ATA	CTT	GAG	TGG	TGT	GTG	TTA	TCA	GAT	AAA	GCA	CCC
Val	Lys	Lys	Tyr	Ile	Leu	Glu	Trp	Cys	Val	Leu	Ser	Asp	Lys	Ala	Pro
2360	2370	2380	2390	2400											

Figure 24E

* * * * *

TGT ATC ACA GAC TGG CAA CAA GAA GAT GGT ACC GTG CAT CGC ACC TAT
Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly Thr Val His Arg Thr Tyr>

2410	2420	2430	2440
*	*	*	*
TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC TAT TTG ATA ACA GTT ACT Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys Tyr Leu Thr Val Thr>			

2450	2460	2470	2480	2490
*	*	*	*	*
CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT GAA TCC ATA AAG GCA TAC Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro Glu Ser Ile Lys Ala Tyr>				*

2500	2510	2520	2530	2540
*	*	*	*	*
CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT ACT GTT CGG ACA AAA AAA Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro Thr Val Arg Thr Lys Lys>				

2550	2560	2570	2580	2590
*	*	*	*	*
GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG GAC CAA CTT CCT GTT GAT Val Gly Lys Asn Glu Ala Val Leu Glu Trp Asp Gln Leu Pro Val Asp>				

2600	2610	2620	2630	2640
*	*	*	*	*
GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT ATA TTT TAT AGA ACC ATC Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr Ile Phe Tyr Arg Thr Ile>				

2650	2660	2670	2680	
*	*	*	*	
ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT TCT TCC CAC ACA GAA TAT Ile Gly Asn Glu Thr Ala Val Asn Val Asp Ser Ser His Thr Glu Tyr>				

2690	2700	2710	2720	2730
*	*	*	*	*
ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG TAC ATG GTA CGA ATG GCA Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu Tyr Met Val Arg Met Ala>				

2740	2750	2760	2770	2780
*	*	*	*	*
GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT CCA GAA TTC ACT TTT ACT Ala Tyr Thr Asp Glu Gly Lys Asp Gly Pro Glu Phe Thr Phe Thr>				

2790	2800	2810	2820	2830
*	*	*	*	*
ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA TCC GGG GGC GAC AAA ACT Thr Pro Lys Phe Ala Gln Gly Glu Ser Gly Asp Lys Thr>				

2840	2850	2860	2870	2880
*	*	*	*	*
CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser>				

2890	2900	2910	2920	
*	*	*	*	
GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg>				

2930	2940	2950	2960	2970
*	*	*	*	*
ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT				

Figure 24F

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro>

2980	2990	3000	3010	3020
* * *	* * *	* * *	* * *	* * *
GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC				
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala>				
3030	3040	3050	3060	3070
* * *	* * *	* * *	* * *	* * *
AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC				
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val>				
3080	3090	3100	3110	3120
* * *	* * *	* * *	* * *	* * *
AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC				
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr>				
3130	3140	3150	3160	
* * *	* * *	* * *	* * *	
AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC				
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr>				
3170	3180	3190	3200	3210
* * *	* * *	* * *	* * *	* * *
ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG				
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu>				
3220	3230	3240	3250	3260
* * *	* * *	* * *	* * *	* * *
CCC CCA TCC CGG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG ACC TGC				
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys>				
3270	3280	3290	3300	3310
* * *	* * *	* * *	* * *	* * *
CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC				
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser>				
3320	3330	3340	3350	3360
* * *	* * *	* * *	* * *	* * *
AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC				
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp>				
3370	3380	3390	3400	
* * *	* * *	* * *	* * *	
TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC				
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser>				
3410	3420	3430	3440	3450
* * *	* * *	* * *	* * *	* * *
AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT				
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala>				
3460	3470	3480	3490	3500
* * *	* * *	* * *	* * *	* * *
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA				
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Pro Gly Lys>				
*				
TGA				
***>				

Figure 25A

	10	20	30	40	
*	*	*	*	*	*
ATG	GTC	GCC	GCG	CCG	CCG
Met	Val	Ala	Val	Gly	Cys
50	60	70	80	90	
*	*	*	*	*	*
GGA	GCG	GCG	CTG	GCC	AGA
Gly	Ala	Ala	Leu	Ala	Arg
100	110	120	130	140	
*	*	*	*	*	*
GGC	GTG	CTG	ACC	AGT	GTG
Gly	Val	Leu	Thr	Ser	Leu
150	160	170	180	190	
*	*	*	*	*	*
GGG	GTA	GAG	CCG	GAA	AAG
Gly	Val	Glu	Pro	Glu	Asn
200	210	220	230	240	
*	*	*	*	*	*
CCG	GCT	GCA	GGC	TCC	AGG
Pro	Ala	Ala	Gly	Ser	His
250	260	270	280		
*	*	*	*	*	*
CTG	CTG	CTG	AGG	TCG	GTG
Leu	Leu	Leu	Arg	Ser	Val
290	300	310	320	330	
*	*	*	*	*	*
TAC	CGG	GCC	GGC	CGC	CAC
Tyr	Arg	Ala	Gly	Arg	Pro
340	350	360	370	380	
*	*	*	*	*	*
CCC	CCC	GAG	GAG	CCC	CAG
Pro	Pro	Glu	Glu	Pro	Gln
390	400	410	420	430	
*	*	*	*	*	*
AAT	GTT	GTT	TGT	GAG	TGG
Asn	Val	Val	Cys	Glu	Trp
440	450	460	470	480	
*	*	*	*	*	*
AAG	GCT	GTG	CTC	TTG	GTG
Lys	Ala	Val	Leu	Leu	Arg
490	500	510	520		
*	*	*	*	*	*
TTC	CAG	GAG	CCG	TGC	CAG
Phe	Gln	Glu	Pro	Cys	Gln
530	540	550	560	570	
*	*	*	*	*	*
CAG	TTA	GCA	GTC	CCG	GAG
Gln	Leu	Ala	Val	Pro	Glu

Figure 25B

580	590	600	610	620
*	*	*	*	*
TGC GTC GCC AGT AGT GTC GGG AGC AAG TTC AGC AAA ACT CAA ACC TTT Cys Val Ala Ser Ser Val Gly Ser Lys Phe Ser Lys Thr Gln Thr Phe>				
630	640	650	660	670
*	*	*	*	*
CAG GGT TGT GGA ATC TTG CAG CCT GAT CCG CCT GCC AAC ATC ACA GTC Gln Gly Cys Gly Ile Leu Gln Pro Asp Pro Pro Ala Asn Ile Thr Val>				
680	690	700	710	720
*	*	*	*	*
ACT GCC GTG GCC AGA AAC CCC CGC TGG CTC AGT GTC ACC TGG CAA GAC Thr Ala Val Ala Arg Asn Pro Arg Trp Leu Ser Val Thr Trp Gln Asp>				
730	740	750	760	
*	*	*	*	*
CCC CAC TCC TGG AAC TCA TCT TTC TAC AGA CTA CGG TTT GAG CTC AGA Pro His Ser Trp Asn Ser Phe Tyr Arg Leu Arg Phe Glu Leu Arg>				
770	780	790	800	810
*	*	*	*	*
TAT CGG GCT GAA CGG TCA AAG ACA TTC ACA ACA TGG ATG GTC AAG GAC Tyr Arg Ala Glu Arg Ser Lys Thr Phe Thr Trp Met Val Lys Asp>				
820	830	840	850	860
*	*	*	*	*
CTC CAG CAT CAC TGT GTC ATC CAC GAC GCC TGG AGC GGC CTG AGG CAC Leu Gln His His Cys Val Ile His Asp Ala Trp Ser Gly Leu Arg His>				
870	880	890	900	910
*	*	*	*	*
GTG GTG CAG CTT CGT GCC CAG GAG GAG TTC GGG CAA GGC GAG TGG AGC Val Val Gln Leu Arg Ala Gln Glu Phe Gly Gln Gly Glu Trp Ser>				
920	930	940	950	960
*	*	*	*	*
GAG TGG AGC CCG GAG GCC ATG GGC ACG CCT TGG ACA GAA TCG CGA TCG Glu Trp Ser Pro Glu Ala Met Gly Thr Pro Trp Thr Glu Ser Arg Ser>				
970	980	990	1000	
*	*	*	*	*
CCT CCA GCT GAG AAC GAG GTG TCC ACC CCC ATG GAA CTT CTA GAC CCA Pro Pro Ala Glu Asn Glu Val Ser Thr Pro Met Glu Leu Leu Asp Pro>				
1010	1020	1030	1040	1050
*	*	*	*	*
TGT GGT TAT ATC AGT CCT GAA TCT CCA GTT GTA CAA CTT CAT TCT AAT Cys Gly Tyr Ile Ser Pro Glu Ser Pro Val Val Gln Leu His Ser Asn>				
1060	1070	1080	1090	1100
*	*	*	*	*
TTC ACT GCA GTT TGT GTG CTA AAG GAA AAA TGT ATG GAT TAT TTT CAT Phe Thr Ala Val Cys Val Leu Lys Glu Lys Cys Met Asp Tyr Phe His>				
1110	1120	1130	1140	1150
*	*	*	*	*
GTA AAT GCT AAT TAC ATT GTC TGG AAA ACA AAC CAT TTT ACT ATT CCT Val Asn Ala Asn Tyr Ile Val Trp Lys Thr Asn His Phe Thr Ile Pro>				
1160	1170	1180	1190	1200
*	*	*	*	*

Figure 25C

AAG GAG CAA TAT ACT ATC ATA AAC AGA ACA GCA TCC AGT GTC ACC TTT
 Lys Glu Gln Tyr Thr Ile Ile Asn Arg Thr Ala Ser Ser Val Thr Phe>

	1210	1220	1230	1240												
*	*	*	*	*	*											
ACA	GAT	ATA	GCT	TCA	TTA	AAT	ATT	CAG	CTC	ACT	TGC	AAC	ATT	CTT	ACA	
Thr	Asp	Ile	Ala	Ser	Leu	Asn	Ile	Gln	Leu	Thr	Cys	Asn	Ile	Leu	Thr	

1250	1260	1270	1280	1290												
*	*	*	*	*	*											
TTC	GGA	CAG	CTT	GAA	CAG	AAT	GTT	TAT	GGA	ATC	ACA	ATA	ATT	TCA	GGC	
Phe	Gly	Gln	Leu	Glu	Gln	Asn	Val	Tyr	Gly	Ile	Thr	Ile	Ile	Ser	Gly	>

	1300	1310	1320	1330	1340											
*	*	*	*	*	*	*										
TTG	CCT	CCA	GAA	AAA	CCT	AAA	AAT	TTG	AGT	TGC	ATT	GTG	AAC	GAG	GGG	
Leu	Pro	Pro	Glu	Lys	Pro	Asn	Leu	Ser	Cys	Ile	Val	Asn	Glu	Gly	>	

	1350	1360	1370	1380	1390											
*	*	*	*	*	*	*										
AAG	AAA	ATG	AGG	TGT	GAG	TGG	GAT	GGT	GGA	AGG	GAA	ACA	CAC	TTG	GAG	
Lys	Lys	Met	Arg	Cys	Glu	Trp	Asp	Gly	Gly	Arg	Glu	Thr	His	Leu	Glu	>

	1400	1410	1420	1430	1440											
*	*	*	*	*	*	*										
ACA	AAC	TTC	ACT	TTA	AAA	TCT	GAA	TGG	GCA	ACA	CAC	AAG	TTT	GCT	GAT	
Thr	Asn	Phe	Thr	Leu	Lys	Ser	Glu	Trp	Ala	Thr	His	Lys	Phe	Ala	Asp	>

	1450	1460	1470	1480												
*	*	*	*	*	*											
TGC	AAA	GCA	AAA	CGT	GAC	ACC	CCC	ACC	TCA	TGC	ACT	GTT	GAT	TAT	TCT	
Cys	Lys	Ala	Lys	Arg	Asp	Thr	Pro	Thr	Ser	Cys	Thr	Val	Asp	Tyr	Ser	>

1490	1500	1510	1520	1530												
*	*	*	*	*	*											
ACT	GTG	TAT	TTT	GTC	AAC	ATT	GAA	GTC	TGG	GTA	GAA	GCA	GAG	AAT	GCC	
Thr	Val	Tyr	Phe	Val	Asn	Ile	Glu	Val	Trp	Val	Glu	Ala	Glu	Asn	Ala	>

	1540	1550	1560	1570	1580											
*	*	*	*	*	*	*										
CTT	GGG	AAG	GTT	ACA	TCA	GAT	CAT	ATC	AAT	TTT	GAT	CCT	GTA	TAT	AAA	
Leu	Gly	Lys	Val	Thr	Ser	Asp	His	Ile	Asn	Phe	Asp	Pro	Val	Tyr	Lys	>

	1590	1600	1610	1620	1630											
*	*	*	*	*	*	*										
GTG	AAG	CCC	AAT	CCG	CCA	CAT	AAT	TTA	TCA	GTG	ATC	AAC	TCA	GAG	GAA	
Val	Lys	Pro	Asn	Pro	Pro	His	Asn	Leu	Ser	Val	Ile	Asn	Ser	Glu	Glu	>

	1640	1650	1660	1670	1680											
*	*	*	*	*	*	*										
CTG	TCT	AGT	ATC	TTA	AAA	TTG	ACA	TGG	ACC	AAC	CCA	AGT	ATT	AAG	AGT	
Leu	Ser	Ser	Ile	Leu	Lys	Leu	Thr	Trp	Thr	Asn	Pro	Ser	Ile	Lys	Ser	>

	1690	1700	1710	1720												
*	*	*	*	*	*											
GTT	ATA	ATA	CTA	AAA	TAT	AAC	ATT	CAA	TAT	AGG	ACC	AAA	GAT	GCC	TCA	
Val	Ile	Ile	Leu	Lys	Tyr	Asn	Ile	Gln	Tyr	Arg	Thr	Lys	Asp	Ala	Ser	>

1730	1740	1750	1760	1770												
*	*	*	*	*	*											
ACT	TGG	AGC	CAG	ATT	CCT	CCT	GAA	GAC	ACA	GCA	TCC	ACC	CGA	TCT	TCA	
Thr	Trp	Ser	Gln	Ile	Pro	Pro	Glu	Asp	Thr	Ala	Ser	Thr	Arg	Ser	Ser	>

Figure 25D

1780	1790	1800	1810	1820
*	*	*	*	*
TTC ACT GTC CAA GAC CTT AAA CCT TTT ACA GAA TAT GTG TTT AGG ATT				
Phe Thr Val Gln Asp Leu Lys Pro Phe Thr Glu Tyr Val Phe Arg Ile>				
1830	1840	1850	1860	1870
*	*	*	*	*
CGC TGT ATG AAG GAA GAT GGT AAG GGA TAC TGG AGT GAC TGG AGT GAA				
Arg Cys Met Lys Glu Asp Gly Lys Gly Tyr Trp Ser Asp Trp Ser Glu>				
1880	1890	1900	1910	1920
*	*	*	*	*
GAA GCA AGT GGG ATC ACC TAT GAA GAT AGA CCA TCT AAA GCA CCA AGT				
Glu Ala Ser Gly Ile Thr Tyr Glu Asp Arg Pro Ser Lys Ala Pro Ser>				
1930	1940	1950	1960	
*	*	*	*	
TTC TGG TAT AAA ATA GAT CCA TCC CAT ACT CAA GGC TAC AGA ACT GTA				
Phe Trp Tyr Lys Ile Asp Pro Ser His Thr Gln Gly Tyr Arg Thr Val>				
1970	1980	1990	2000	2010
*	*	*	*	*
CAA CTC GTG TGG AAG ACA TTG CCT CCT TTT GAA GCC AAT GGA AAA ATC				
Gln Leu Val Trp Lys Thr Leu Pro Pro Phe Glu Ala Asn Gly Lys Ile>				
2020	2030	2040	2050	2060
*	*	*	*	*
TTG GAT TAT GAA GTG ACT CTC ACA AGA TGG AAA TCA CAT TTA CAA AAT				
Leu Asp Tyr Glu Val Thr Leu Thr Arg Trp Lys Ser His Leu Gln Asn>				
2070	2080	2090	2100	2110
*	*	*	*	*
TAC ACA GTT AAT GCC ACA AAA CTG ACA GTA AAT CTC ACA AAT GAT CGC				
Tyr Thr Val Asn Ala Thr Lys Leu Thr Val Asn Leu Thr Asn Asp Arg>				
2120	2130	2140	2150	2160
*	*	*	*	*
TAT CTA GCA ACC CTA ACA GTA AGA AAT CTT GTT GGC AAA TCA GAT GCA				
Tyr Leu Ala Thr Leu Thr Val Arg Asn Leu Val Gly Lys Ser Asp Ala>				
2170	2180	2190	2200	
*	*	*	*	
GCT GTT TTA ACT ATC CCT GCC TGT GAC TTT CAA GCT ACT CAC CCT GTA				
Ala Val Leu Thr Ile Pro Ala Cys Asp Phe Gln Ala Thr His Pro Val>				
2210	2220	2230	2240	2250
*	*	*	*	*
ATG GAT CTT AAA GCA TTC CCC AAA GAT AAC ATG CTT TGG GTG GAA TGG				
Met Asp Leu Lys Ala Phe Pro Lys Asp Asn Met Leu Trp Val Glu Trp>				
2260	2270	2280	2290	2300
*	*	*	*	*
ACT ACT CCA AGG GAA TCT GTA AAG AAA TAT ATA CTT GAG TGG TGT GTG				
Thr Thr Pro Arg Glu Ser Val Lys Lys Tyr Ile Leu Glu Trp Cys Val>				
2310	2320	2330	2340	2350
*	*	*	*	*
TTA TCA GAT AAA GCA CCC TGT ATC ACA GAC TGG CAA CAA GAA GAT GGT				
Leu Ser Asp Lys Ala Pro Cys Ile Thr Asp Trp Gln Gln Glu Asp Gly>				
2360	2370	2380	2390	2400

Figure 25E

* * * * *

ACC GTG CAT CGC ACC TAT TTA AGA GGG AAC TTA GCA GAG AGC AAA TGC
Thr Val His Arg Thr Tyr Leu Arg Gly Asn Leu Ala Glu Ser Lys Cys>

2410	2420	2430	2440
*	*	*	*

TAT TTG ATA ACA GTT ACT CCA GTA TAT GCT GAT GGA CCA GGA AGC CCT
Tyr Leu Ile Thr Val Thr Pro Val Tyr Ala Asp Gly Pro Gly Ser Pro>

2450	2460	2470	2480	2490
*	*	*	*	*

GAA TCC ATA AAG GCA TAC CTT AAA CAA GCT CCA CCT TCC AAA GGA CCT
Glu Ser Ile Lys Ala Tyr Leu Lys Gln Ala Pro Pro Ser Lys Gly Pro>

2500	2510	2520	2530	2540
*	*	*	*	*

ACT GTT CGG ACA AAA AAA GTA GGG AAA AAC GAA GCT GTC TTA GAG TGG
Thr Val Arg Thr Lys Lys Val Gly Lys Asn Glu Ala Val Leu Glu Trp>

2550	2560	2570	2580	2590
*	*	*	*	*

GAC CAA CTT CCT GTT GAT GTT CAG AAT GGA TTT ATC AGA AAT TAT ACT
Asp Gln Leu Pro Val Asp Val Gln Asn Gly Phe Ile Arg Asn Tyr Thr>

2600	2610	2620	2630	2640
*	*	*	*	*

ATA TTT TAT AGA ACC ATC ATT GGA AAT GAA ACT GCT GTG AAT GTG GAT
Ile Phe Tyr Arg Thr Ile Ile Gly Asn Glu Thr Ala Val Asn Val Asp>

2650	2660	2670	2680
*	*	*	*

TCT TCC CAC ACA GAA TAT ACA TTG TCC TCT TTG ACT AGT GAC ACA TTG
Ser Ser His Thr Glu Tyr Thr Leu Ser Ser Leu Thr Ser Asp Thr Leu>

2690	2700	2710	2720	2730
*	*	*	*	*

TAC ATG GTA CGA ATG GCA GCA TAC ACA GAT GAA GGT GGG AAG GAT GGT
Tyr Met Val Arg Met Ala Ala Tyr Thr Asp Glu Gly Lys Asp Gly>

2740	2750	2760	2770	2780
*	*	*	*	*

CCA GAA TTC ACT TTT ACT ACC CCA AAG TTT GCT CAA GGA GAA ATT GAA
Pro Glu Phe Thr Phe Thr Pro Lys Phe Ala Gln Gly Glu Ile Glu>

2790	2800	2810	2820	2830
*	*	*	*	*

TCC GGG GGC GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA
Ser Gly Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu>

2840	2850	2860	2870	2880
*	*	*	*	*

CTC CTG GGG GGA CCG TCA GTC TTC CTC CCC CCA AAA CCC AAG GAC
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp>

2890	2900	2910	2920
*	*	*	*

ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp>

2930	2940	2950	2960	2970
*	*	*	*	*

GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC

Figure 25F

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly>

2980	2990	3000	3010	3020
* * *	* * *	* * *	* * *	* * *
GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC				
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn>				
3030	3040	3050	3060	3070
* * *	* * *	* * *	* * *	* * *
AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG				
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp>				
3080	3090	3100	3110	3120
* * *	* * *	* * *	* * *	* * *
CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA				
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro>				
3130	3140	3150	3160	
* * *	* * *	* * *	* * *	
GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA				
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu>				
3170	3180	3190	3200	3210
* * *	* * *	* * *	* * *	* * *
CCA CAG GTG TAC ACC CTG CCC CCA TCC CCG GAT GAG CTG ACC AAG AAC				
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn>				
3220	3230	3240	3250	3260
* * *	* * *	* * *	* * *	* * *
CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC				
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile>				
3270	3280	3290	3300	3310
* * *	* * *	* * *	* * *	* * *
GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC				
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr>				
3320	3330	3340	3350	3360
* * *	* * *	* * *	* * *	* * *
ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG				
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys>				
3370	3380	3390	3400	
* * *	* * *	* * *	* * *	
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC				
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys>				
3410	3420	3430	3440	3450
* * *	* * *	* * *	* * *	* * *
TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC				
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu>				
3460	3470			
* * *	* * *			
TCC CTG TCT CCG GGT AAA TGA				
Ser Leu Ser Pro Gly Lys ***>				

Figure 26A

	10	20	30	40												
*	*	*	*	*	*											
ATG	GTG	CTT	CTG	TGG	TGT	GTA	GTG	AGT	CTC	TAC	TTT	TAT	GGA	ATC	CTG	
Met	Val	Leu	Leu	Trp	Cys	Val	Val	Ser	Leu	Tyr	Phe	Tyr	Gly	Ile	Leu	>
	50	60	70	80	90											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CAA	AGT	GAT	GCC	TCA	GAA	CGC	TGC	GAT	GAC	TGG	GGA	CTA	GAC	ACC	ATG	
Gln	Ser	Asp	Ala	Ser	Glu	Arg	Cys	Asp	Asp	Trp	Gly	Leu	Asp	Thr	Met	>
	100	110	120	130	140											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
AGG	CAA	ATC	CAA	GTG	TTT	GAA	GAT	GAG	CCA	GCT	CGC	ATC	AAG	TGC	CCA	
Arg	Gln	Ile	Gln	Val	Phe	Glu	Asp	Glu	Pro	Ala	Arg	Ile	Lys	Cys	Pro	>
	150	160	170	180	190											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CTC	TTT	GAA	CAC	TTC	TTG	AAA	TTC	AAC	TAC	AGC	ACA	GCC	CAT	TCA	GCT	
Leu	Phe	Glu	His	Phe	Leu	Lys	Phe	Asn	Tyr	Ser	Thr	Ala	His	Ser	Ala	>
	200	210	220	230	240											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
GGC	CTT	ACT	CTG	ATC	TGG	TAT	TGG	ACT	AGG	CAG	GAC	CGG	GAC	CTT	GAG	
Gly	Leu	Thr	Leu	Ile	Trp	Tyr	Trp	Thr	Arg	Gln	Asp	Arg	Asp	Leu	Glu	>
	250	260	270	280												
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
GAG	CCA	ATT	AAC	TTC	CGC	CTC	CCC	GAG	AAC	CGC	ATT	AGT	AAG	GAG	AAA	
Glu	Pro	Ile	Asn	Phe	Arg	Leu	Pro	Glu	Asn	Arg	Ile	Ser	Lys	Glu	Lys	>
	290	300	310	320	330											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
GAT	GTG	CTG	TGG	TTC	CGG	CCC	ACT	CTC	CTC	AAT	GAC	ACT	GGC	AAC	TAT	
Asp	Val	Leu	Trp	Phe	Arg	Pro	Thr	Leu	Leu	Asn	Asp	Thr	Gly	Asn	Tyr	>
	340	350	360	370	380											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
ACC	TGC	ATG	TTA	AGG	AAC	ACT	ACA	TAT	TGC	AGC	AAA	GTT	GCA	TTT	CCC	
Thr	Cys	Met	Leu	Arg	Asn	Thr	Thr	Tyr	Cys	Ser	Lys	Val	Ala	Phe	Pro	>
	390	400	410	420	430											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
TTG	GAA	GTT	GTT	CAA	AAA	GAC	AGC	TGT	TTC	AAT	TCC	CCC	ATG	AAA	CTC	
Leu	Glu	Val	Val	Gln	Lys	Asp	Ser	Cys	Phe	Asn	Ser	Pro	Met	Lys	Leu	>
	440	450	460	470	480											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CCA	GTG	CAT	AAA	CTG	TAT	ATA	GAA	TAT	GGC	ATT	CAG	AGG	ATC	ACT	TGT	
Pro	Val	His	Lys	Leu	Tyr	Ile	Glu	Tyr	Gly	Ile	Gln	Arg	Ile	Thr	Cys	>
	490	500	510	520												
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
CCA	AAT	GTA	GAT	GGA	TAT	TTT	CCT	TCC	AGT	GTC	AAA	CCG	ACT	ATC	ACT	
Pro	Asn	Val	Asp	Gly	Tyr	Phe	Pro	Ser	Ser	Val	Lys	Pro	Thr	Ile	Thr	>
	530	540	550	560	570											
*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
TGG	TAT	ATG	GGC	TGT	TAT	AAA	ATA	CAG	AAT	TTT	AAT	AAT	GTA	ATA	CCC	
Trp	Tyr	Met	Gly	Cys	Tyr	Lys	Ile	Gln	Asn	Phe	Asn	Asn	Val	Ile	Pro	>

Figure 26B

580	590	600	610	620
*	*	*	*	*
GAA GGT ATG AAC TTG AGT TTC CTC ATT GCC TTA ATT TCA AAT AAT GGA				
Glu Gly Met Asn Leu Ser Phe Leu Ile Ala Leu Ile Ser Asn Asn Gly>				
630	640	650	660	670
*	*	*	*	*
AAT TAC ACA TGT GTT ACA TAT CCA GAA AAT GGA CGT ACG TTT CAT				
Asn Tyr Thr Cys Val Val Thr Tyr Pro Glu Asn Gly Arg Thr Phe His>				
680	690	700	710	720
*	*	*	*	*
CTC ACC AGG ACT CTG ACT GTA AAG GTA GTA GGC TCT CCA AAA AAT GCA				
Leu Thr Arg Thr Leu Thr Val Lys Val Val Gly Ser Pro Lys Asn Ala>				
730	740	750	760	
*	*	*	*	
GTG CCC CCT GTG ATC CAT TCA CCT AAT GAT CAT GTG GTC TAT GAG AAA				
Val Pro Pro Val Ile His Ser Pro Asn Asp His Val Val Tyr Glu Lys>				
770	780	790	800	810
*	*	*	*	*
GAA CCA GGA GAG GAG CTA CTC ATT CCC TGT ACG GTC TAT TTT AGT TTT				
Glu Pro Gly Glu Leu Leu Ile Pro Cys Thr Val Tyr Phe Ser Phe>				
820	830	840	850	860
*	*	*	*	*
CTG ATG GAT TCT CGC AAT GAG GTT TGG TGG ACC ATT GAT GGA AAA AAA				
Leu Met Asp Ser Arg Asn Glu Val Trp Trp Thr Ile Asp Gly Lys Lys>				
870	880	890	900	910
*	*	*	*	*
CCT GAT GAC ATC ACT ATT GAT GTC ACC ATT AAC GAA AGT ATA AGT CAT				
Pro Asp Asp Ile Thr Ile Asp Val Thr Ile Asn Glu Ser Ile Ser His>				
920	930	940	950	960
*	*	*	*	*
AGT AGA ACA GAA GAT GAA ACA AGA ACT CAG ATT TTG AGC ATC AAG AAA				
Ser Arg Thr Glu Asp Glu Thr Arg Thr Gln Ile Leu Ser Ile Lys Lys>				
970	980	990	1000	
*	*	*	*	
GTT ACC TCT GAG GAT CTC AAG CGC AGC TAT GTC TGT CAT GCT AGA AGT				
Val Thr Ser Glu Asp Leu Lys Arg Ser Tyr Val Cys His Ala Arg Ser>				
1010	1020	1030	1040	1050
*	*	*	*	*
GCC AAA GGC GAA GTT GCC AAA GCA GCC AAG GTG AAG CAG AAA GTG CCA				
Ala Lys Gly Glu Val Ala Lys Ala Ala Lys Val Lys Gln Lys Val Pro>				
1060	1070	1080	1090	1100
*	*	*	*	*
GCT CCA AGA TAC ACA GTG TCC GGT GGC GCG CCT ATG CTG AGC GAG GCT				
Ala Pro Arg Tyr Thr Val Ser Gly Gly Ala Pro Met Leu Ser Glu Ala>				
1110	1120	1130	1140	1150
*	*	*	*	*
GAT AAA TGC AAG GAA CGT GAA GAA AAA ATA ATT TTA GTG TCA TCT GCA				
Asp Lys Cys Lys Glu Arg Glu Glu Lys Ile Ile Leu Val Ser Ser Ala>				
1160	1170	1180	1190	1200
*	*	*	*	*

Figure 26C

AAT GAA ATT GAT GTT CGT CCC TGT CCT CTT AAC CCA AAT GAA CAC AAA
Asn Glu Ile Asp Val Arg Pro Cys Pro Leu Asn Pro Asn Glu His Lys>

1210	1220	1230	1240	
*	*	*	*	
GGC ACT ATA ACT TGG TAT AAG GAT GAC AGC AAG ACA CCT GTA TCT ACA				
Gly Thr Ile Thr Trp Tyr Lys Asp Asp Ser Lys Thr Pro Val Ser Thr>				
1250	1260	1270	1280	1290
*	*	*	*	*
GAA CAA GCC TCC AGG ATT CAT CAA CAC AAA GAG AAA CTT TGG TTT GTT				
Glu Gln Ala Ser Arg Ile His Gln His Lys Glu Lys Leu Trp Phe Val>				
1300	1310	1320	1330	1340
*	*	*	*	*
CCT GCT AAG GTG GAG GAT TCA GGA CAT TAC TAT TGC GTG GTA AGA AAT				
Pro Ala Lys Val Glu Asp Ser Gly His Tyr Tyr Cys Val Val Arg Asn>				
1350	1360	1370	1380	1390
*	*	*	*	*
TCA TCT TAC TGC CTC AGA ATT AAA ATA AGT GCA AAA TTT GTG GAG AAT				
Ser Ser Tyr Cys Leu Arg Ile Lys Ile Ser Ala Lys Phe Val Glu Asn>				
1400	1410	1420	1430	1440
*	*	*	*	*
GAG CCT AAC TTA TGT TAT AAT GCA CAA GCC ATA TTT AAG CAG AAA CTA				
Glu Pro Asn Leu Cys Tyr Asn Ala Gln Ala Ile Phe Lys Gln Lys Leu>				
1450	1460	1470	1480	
*	*	*	*	*
CCC GTT GCA GGA GAC GGA GGA CTT GTG TGC CCT TAT ATG GAG TTT TTT				
Pro Val Ala Gly Asp Gly Leu Val Cys Pro Tyr Met Glu Phe Phe>				
1490	1500	1510	1520	1530
*	*	*	*	*
AAA AAT GAA AAT AAT GAG TTA CCT AAA TTA CAG TGG TAT AAG GAT TGC				
Lys Asn Glu Asn Asn Glu Leu Pro Lys Leu Gln Trp Tyr Lys Asp Cys>				
1540	1550	1560	1570	1580
*	*	*	*	*
AAA CCT CTA CTT CTT GAC AAT ATA CAC TTT AGT GGA GTC AAA GAT AGG				
Lys Pro Leu Leu Leu Asp Asn Ile His Phe Ser Gly Val Lys Asp Arg>				
1590	1600	1610	1620	1630
*	*	*	*	*
CTC ATC GTG ATG AAT GTG GCT GAA AAG CAT AGA GGG AAC TAT ACT TGT				
Leu Ile Val Met Asn Val Ala Glu Lys His Arg Gly Asn Tyr Thr Cys>				
1640	1650	1660	1670	1680
*	*	*	*	*
CAT GCA TCC TAC ACA TAC TTG GGC AAG CAA TAT CCT ATT ACC CGG GTA				
His Ala Ser Tyr Thr Tyr Leu Gly Lys Gln Tyr Pro Ile Thr Arg Val>				
1690	1700	1710	1720	
*	*	*	*	*
ATA GAA TTT ATT ACT CTA GAG GAA AAC AAA CCC ACA AGG CCT GTG ATT				
Ile Glu Phe Ile Thr Leu Glu Glu Asn Lys Pro Thr Arg Pro Val Ile>				
1730	1740	1750	1760	1770
*	*	*	*	*
GTG AGC CCA GCT AAT GAG ACA ATG GAA GTA GAC TTG GGA TCC CAG ATA				
Val Ser Pro Ala Asn Glu Thr Met Glu Val Asp Leu Gly Ser Gln Ile>				

Figure 26D

1780	1790	1800	1810	1820
*	*	*	*	*
CAA TTG ATC TGT AAT GTC ACC GGC CAG TTG AGT GAC ATT GCT TAC TGG Gln Leu Ile Cys Asn Val Thr Gly Gln Leu Ser Asp Ile Ala Tyr Trp>				
1830	1840	1850	1860	1870
*	*	*	*	*
AAG TGG AAT GGG TCA GTA ATT GAT GAA GAT GAC CCA GTG CTA GGG GAA Lys Trp Asn Gly Ser Val Ile Asp Glu Asp Asp Pro Val Leu Gly Glu>				
1880	1890	1900	1910	1920
*	*	*	*	*
GAC TAT TAC AGT GTG GAA AAT CCT GCA AAC AAA AGA AGG AGT ACC CTC Asp Tyr Tyr Ser Val Glu Asn Pro Ala Asn Lys Arg Arg Ser Thr Leu>				
1930	1940	1950	1960	
*	*	*	*	*
ATC ACA GTG CTT AAT ATA TCG GAA ATT GAG AGT AGA TTT TAT AAA CAT Ile Thr Val Leu Asn Ile Ser Glu Ile Glu Ser Arg Phe Tyr Lys His>				
1970	1980	1990	2000	2010
*	*	*	*	*
CCA TTT ACC TGT TTT GCC AAG AAT ACA CAT GGT ATA GAT GCA GCA TAT Pro Phe Thr Cys Phe Ala Lys Asn Thr His Gly Ile Asp Ala Ala Tyr>				
2020	2030	2040	2050	2060
*	*	*	*	*
ATC CAG TTA ATA TAT CCA GTC ACT AAT TCC GGA GAC AAA ACT CAC ACA Ile Gln Leu Ile Tyr Pro Val Thr Asn Ser Gly Asp Lys Thr His Thr>				
2070	2080	2090	2100	2110
*	*	*	*	*
TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe>				
2120	2130	2140	2150	2160
*	*	*	*	*
CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro>				
2170	2180	2190	2200	
*	*	*	*	*
GAG GTC ACA TGC GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC Glu Val Thr Cys Val Val Asp Val Ser His Glu Asp Pro Glu Val>				
2210	2220	2230	2240	2250
*	*	*	*	*
AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr>				
2260	2270	2280	2290	2300
*	*	*	*	*
AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val>				
2310	2320	2330	2340	2350
*	*	*	*	*
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys>				
2360	2370	2380	2390	2400

Figure 26E

* * * * *

AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser>

2410	2420	2430	2440
*	*	*	*

AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro>

2450	2460	2470	2480	2490
*	*	*	*	*

TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC
Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val>

2500	2510	2520	2530	2540
*	*	*	*	*

AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly>

2550	2560	2570	2580	2590
*	*	*	*	*

CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC
Gln Pro Glu Asn Asn Tyr Lys Thr Pro Pro Val Leu Asp Ser Asp>

2600	2610	2620	2630	2640
*	*	*	*	*

GGC TCC TTC TTC CTC TAT AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp>

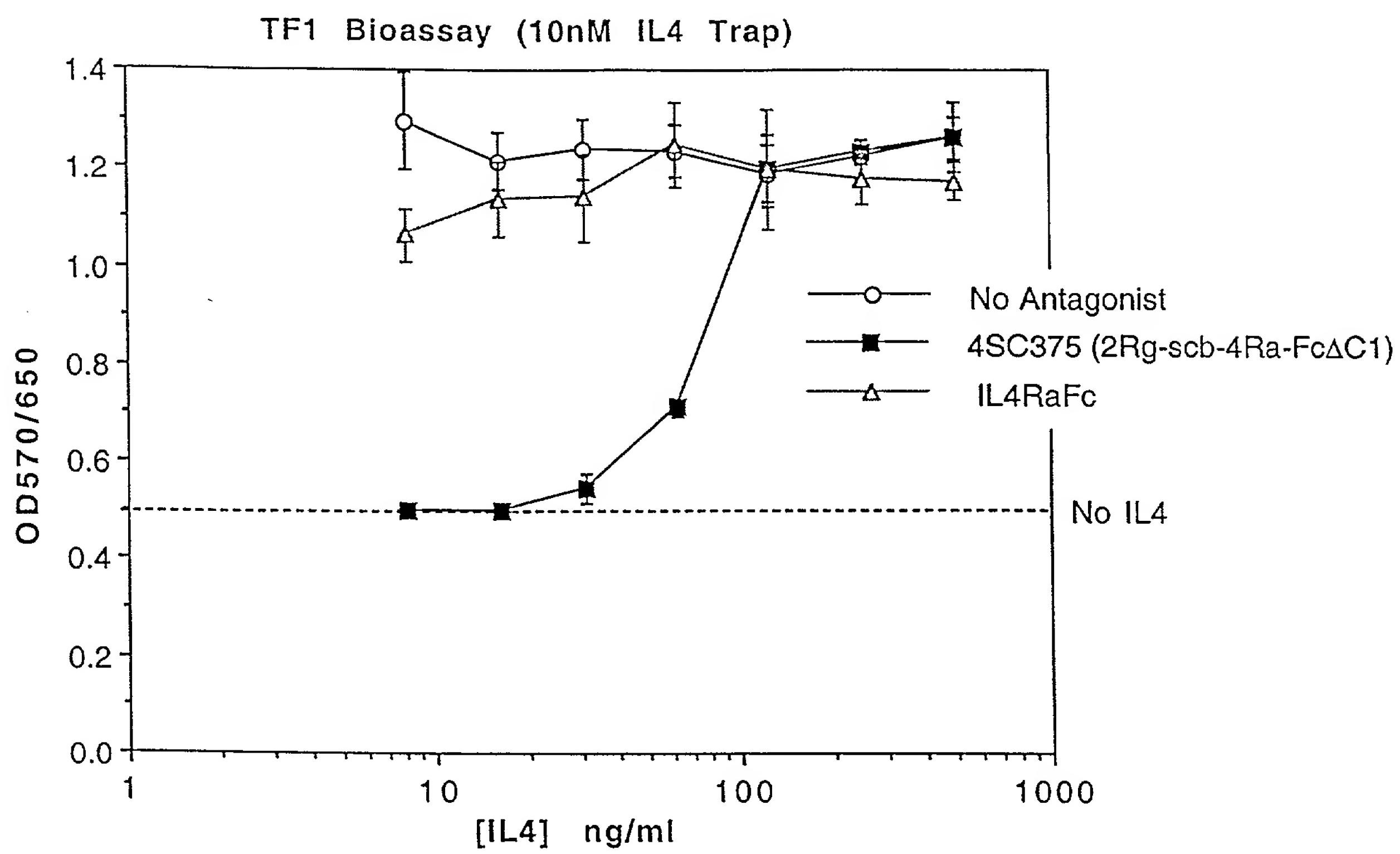
2650	2660	2670	2680
*	*	*	*

CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC
Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His>

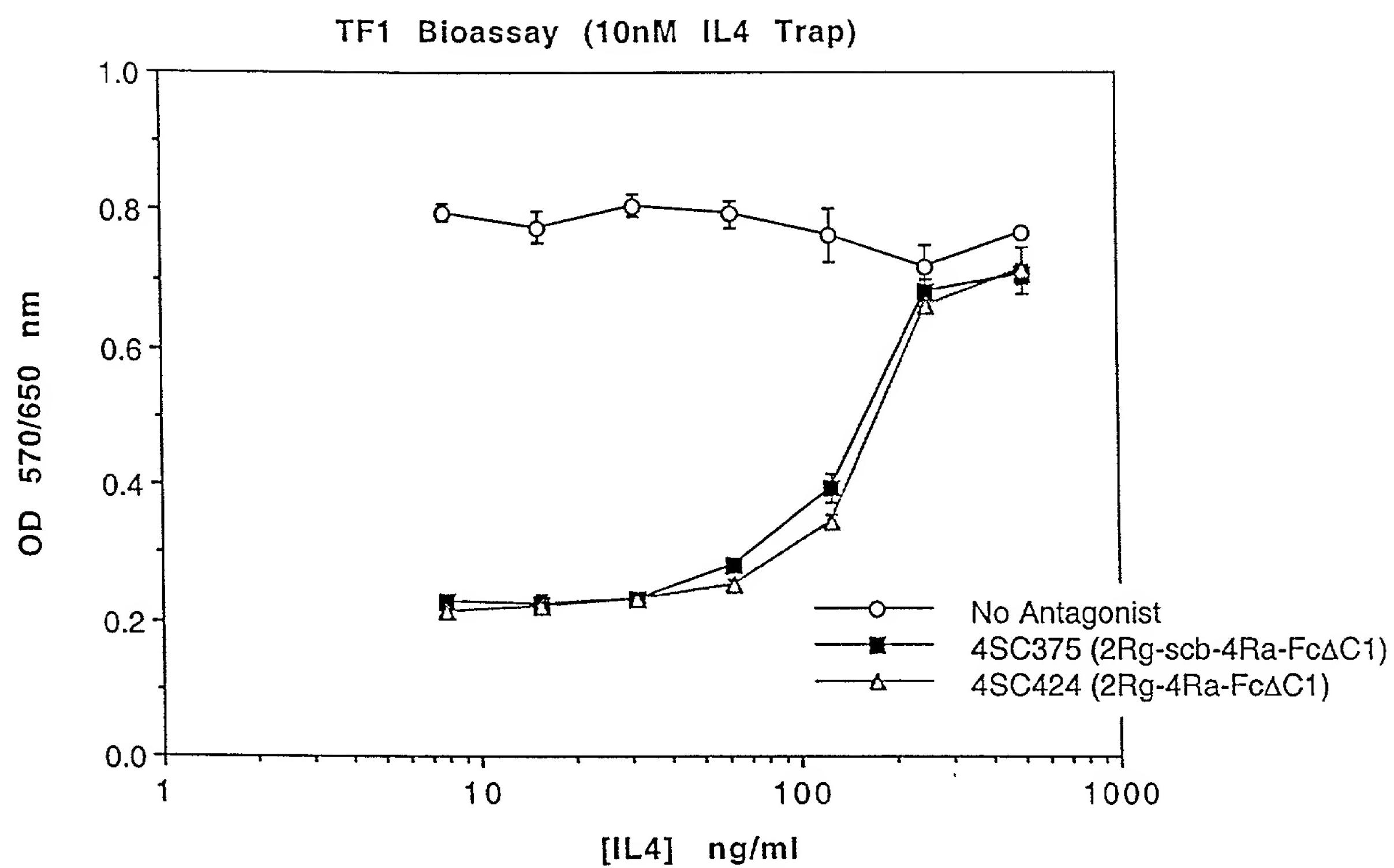
2690	2700	2710	2720	2730
*	*	*	*	*

AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>

52/69
Figure 27



53/69
Figure 28



54/69
Figure 29

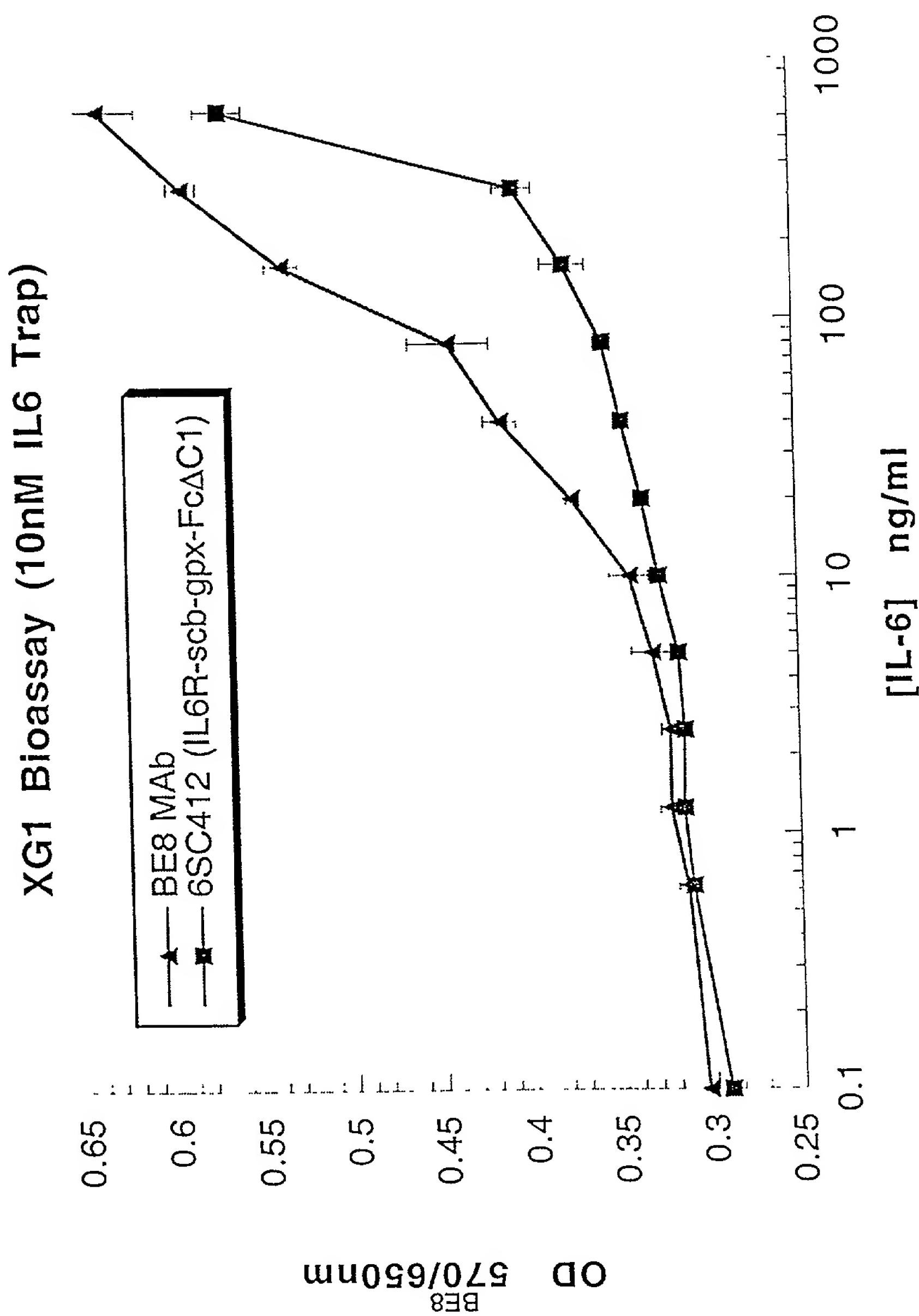


Figure 30

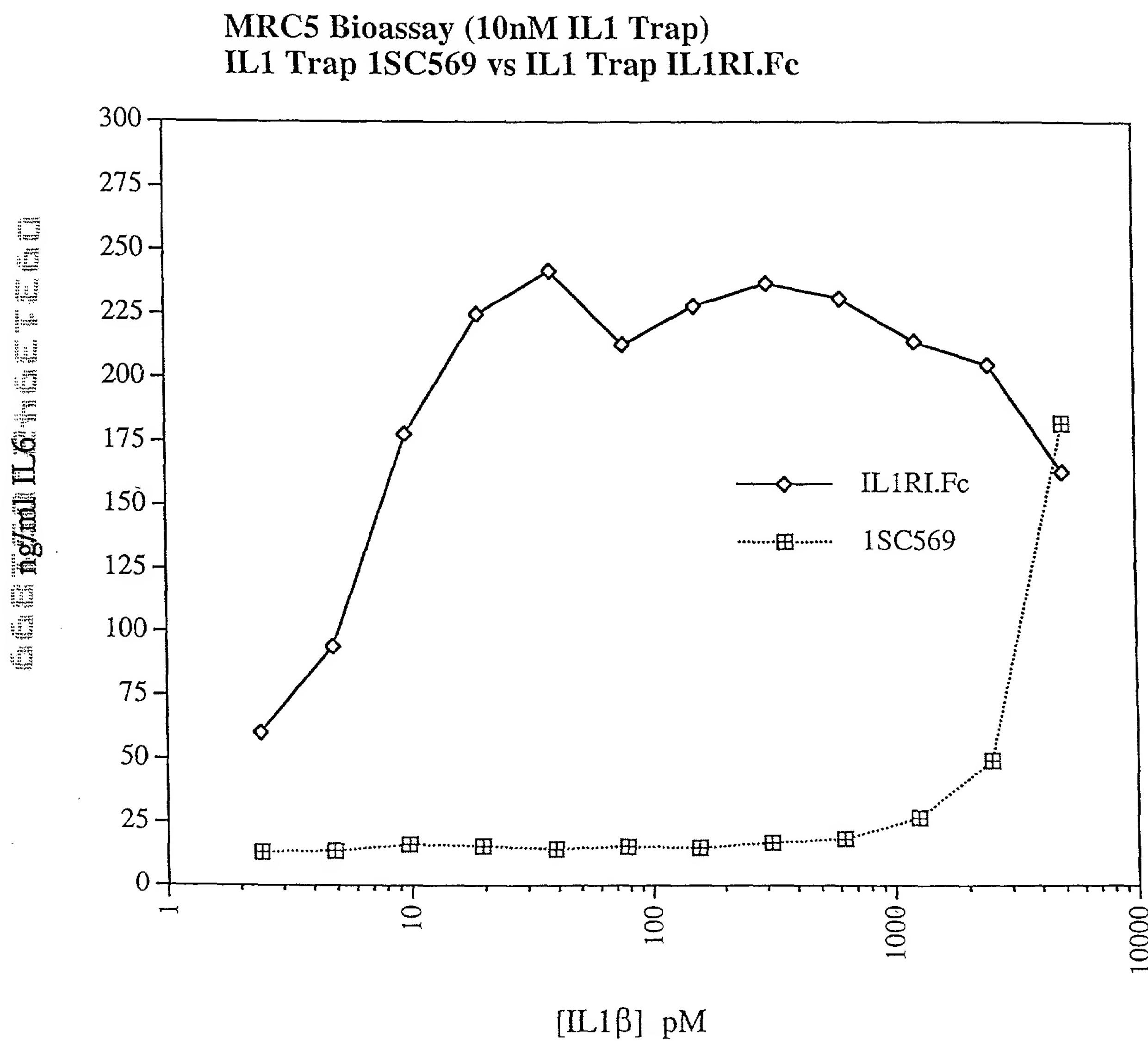


Figure 31A

	10	20	30	40	
*	*	*	*	*	*
ATG GTG TGG CTT TGC TCT GGG CTC CTG TTC CCT GTG AGC TGC CTG GTC					
TAC CAC ACC GAA ACG AGA CCC GAG GAC AAG GGA CAC TCG ACG GAC CAG					
Met Val Trp Leu Cys Ser Gly Leu Leu Phe Pro Val Ser Cys Leu Val>					
	50	60	70	80	90
*	*	*	*	*	*
CTG CTG CAG GTG GCA AGC TCT GGG AAC ATG AAG GTC TTG CAG GAG CCC					
GAC GAC GTC CAC CGT TCG AGA CCC TTG TAC TTC CAG AAC GTC CTC GGG					
Leu Leu Gln Val Ala Ser Ser Gly Asn Met Lys Val Leu Gln Glu Pro>					
	100	110	120	130	140
*	*	*	*	*	*
ACC TGC GTC TCC GAC TAC ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG					
TGG ACG CAG AGG CTG ATG TAC TCG TAG AGA TGA ACG CTC ACC TTC TAC					
Thr Cys Val Ser Asp Tyr Met Ser Ile Ser Thr Cys Glu Trp Lys Met>					
	150	160	170	180	190
*	*	*	*	*	*
AAT GGT CCC ACC AAT TGC AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG					
TTA CCA GGG TGG TTA ACG TCG TGG CTC GAG GCG GAC AAC ATG GTC GAC					
Asn Gly Pro Thr Asn Cys Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu>					
	200	210	220	230	240
*	*	*	*	*	*
GTT TTT CTG CTC TCC GAA GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA					
CAA AAA GAC GAG AGG CTT CGG GTG TGC ACA TAG GGA CTC TTG TTG CCT					
Val Phe Leu Leu Ser Glu Ala His Thr Cys Ile Pro Glu Asn Asn Gly>					
	250	260	270	280	
*	*	*	*	*	*
GGC GCG GGG TGC GTG TGC CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG					
CCG CGC CCC ACG CAC ACG GTG GAC GAG TAC CTA CTG CAC CAG TCA CGC					
Gly Ala Gly Cys Val Cys His Leu Leu Met Asp Asp Val Val Ser Ala>					
	290	300	310	320	330
*	*	*	*	*	*
GAT AAC TAT ACA CTG GAC CTG TGG GCT GGG CAG CAG CTG CTG TGG AAG					
CTA TTG ATA TGT GAC CTG GAC ACC CGA CCC GTC GTC GAC GAC ACC TTC					
Asp Asn Tyr Thr Leu Asp Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys>					
	340	350	360	370	380
*	*	*	*	*	*
GGC TCC TTC AAG CCC AGC GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC					
CCG AGG AAG TTC GGG TCG CTC GTA CAC TTT GGG TCC CGG GGT CCT TTG					
Gly Ser Phe Lys Pro Ser Glu His Val Lys Pro Arg Ala Pro Gly Asn>					

Figure 31B

390	400	410	420	430											
*	*	*	*	*											
CTG	ACA	GTT	CAC	ACC	AAT	GTC	TCC	GAC	ACT	CTG	CTG	CTG	ACC	TGG	AGC
GAC	TGT	CAA	GTG	TGG	TTA	CAG	AGG	CTG	TGA	GAC	GAC	GAC	TGG	ACC	TCG
Leu	Thr	Val	His	Thr	Asn	Val	Ser	Asp	Thr	Leu	Leu	Leu	Thr	Trp	Ser>
440	450	460	470	480											
*	*	*	*	*											
AAC	CCG	TAT	CCC	CCT	GAC	AAT	TAC	CTG	TAT	AAT	CAT	CTC	ACC	TAT	GCA
TTG	GGC	ATA	GGG	GGA	CTG	TTA	ATG	GAC	ATA	TTA	GTA	GAG	TGG	ATA	CGT
Asn	Pro	Tyr	Pro	Pro	Asp	Asn	Tyr	Leu	Tyr	Asn	His	Leu	Thr	Tyr	Ala>
490	500	510	520												
*	*	*	*	*											
GTC	AAC	ATT	TGG	AGT	GAA	AAC	GAC	CCG	GCA	GAT	TTC	AGA	ATC	TAT	AAC
CAG	TTG	TAA	ACC	TCA	CTT	TTG	CTG	GGC	CGT	CTA	AAG	TCT	TAG	ATA	TTG
Val	Asn	Ile	Trp	Ser	Glu	Asn	Asp	Pro	Ala	Asp	Phe	Arg	Ile	Tyr	Asn>
530	540	550	560	570											
*	*	*	*	*	*										
GTG	ACC	TAC	CTA	GAA	CCC	TCC	CTC	CGC	ATC	GCA	GCC	AGC	ACC	CTG	AAG
CAC	TGG	ATG	GAT	CTT	GGG	AGG	GAG	GCG	TAG	CGT	CGG	TCG	TGG	GAC	TTC
Val	Thr	Tyr	Leu	Glu	Pro	Ser	Leu	Arg	Ile	Ala	Ala	Ser	Thr	Leu	Lys>
580	590	600	610	620											
*	*	*	*	*	*										
TCT	GGG	ATT	TCC	TAC	AGG	GCA	CGG	GTG	AGG	GCC	TGG	GCT	CAG	AGC	TAT
AGA	CCC	TAA	AGG	ATG	TCC	CGT	GCC	CAC	TCC	CGG	ACC	CGA	GTC	TCG	ATA
Ser	Gly	Ile	Ser	Tyr	Arg	Ala	Arg	Val	Arg	Ala	Trp	Ala	Gln	Ser	Tyr>
630	640	650	660	670											
*	*	*	*	*	*										
AAC	ACC	ACC	TGG	AGT	GAG	TGG	AGC	CCC	AGC	ACC	AAG	TGG	CAC	AAC	TCC
TTG	TGG	TGG	ACC	TCA	CTC	ACC	TCG	GGG	TCG	TGG	TTC	ACC	GTG	TTG	AGG
Asn	Thr	Thr	Trp	Ser	Glu	Trp	Ser	Pro	Ser	Thr	Lys	Trp	His	Asn	Ser>
680	690	700	710	720											
*	*	*	*	*	*										
TAC	AGG	GAG	CCC	TTC	GAG	CAG	TCC	GGT	GGG	GGC	GGG	GGC	GCC	GCG	CCT
ATG	TCC	CTC	GGG	AAG	CTC	GTC	AGG	CCA	CCC	CCG	CCC	CCG	CGG	CGC	GGA
Tyr	Arg	Glu	Pro	Phe	Glu	Gln	Ser	Gly	Gly	Gly	Gly	Gly	Ala	Ala	Pro>
730	740	750	760												
*	*	*	*	*	*										
ACG	GAA	ACT	CAG	CCA	CCT	GTG	ACA	AAT	TTG	AGT	GTC	TCT	GTT	GAA	AAC
TGC	CTT	TGA	GTC	GGT	GGA	CAC	TGT	TTA	AAC	TCA	CAG	AGA	CAA	CTT	TTG
Thr	Glu	Thr	Gln	Pro	Pro	Val	Thr	Asn	Leu	Ser	Val	Ser	Val	Glu	Asn>

Figure 31C

770	780	790	800	810											
*	*	*	*	*											
CTC	TGC	ACA	GTA	ATA	TGG	ACA	TGG	AAT	CCA	CCC	GAG	GGA	GCC	AGC	TCA
GAG	ACG	TGT	CAT	TAT	ACC	TGT	ACC	TTA	GGT	GGG	CTC	CCT	CGG	TCG	AGT
Leu	Cys	Thr	Val	Ile	Trp	Thr	Trp	Asn	Pro	Pro	Glu	Gly	Ala	Ser	Ser>
820	830	840	850	860											
*	*	*	*	*											
AAT	TGT	AGT	CTA	TGG	TAT	TTT	AGT	CAT	TTT	GGC	GAC	AAA	CAA	GAT	AAG
TTA	ACA	TCA	GAT	ACC	ATA	AAA	TCA	GTA	AAA	CCG	CTG	TTT	GTT	CTA	TTC
Asn	Cys	Ser	Leu	Trp	Tyr	Phe	Ser	His	Phe	Gly	Asp	Lys	Gln	Asp	Lys>
870	880	890	900	910											
*	*	*	*	*											
AAA	ATA	GCT	CCG	GAA	ACT	CGT	CGT	TCA	ATA	GAA	GTA	CCC	CTG	AAT	GAG
TTT	TAT	CGA	GGC	CTT	TGA	GCA	GCA	AGT	TAT	CTT	CAT	GGG	GAC	TTA	CTC
Lys	Ile	Ala	Pro	Glu	Thr	Arg	Arg	Ser	Ile	Glu	Val	Pro	Leu	Asn	Glu>
920	930	940	950	960											
*	*	*	*	*											
AGG	ATT	TGT	CTG	CAA	GTG	GGG	TCC	CAG	TGT	AGC	ACC	AAT	GAG	AGT	GAG
TCC	TAA	ACA	GAC	GTT	CAC	CCC	AGG	GTC	ACA	TCG	TGG	TTA	CTC	TCA	CTC
Arg	Ile	Cys	Leu	Gln	Val	Gly	Ser	Gln	Cys	Ser	Thr	Asn	Glu	Ser	Glu>
970	980	990	1000												
*	*	*	*	*											
AAG	CCT	AGC	ATT	TTG	GTT	GAA	AAA	TGC	ATC	TCA	CCC	CCA	GAA	GGT	GAT
TTC	GGA	TCG	TAA	AAC	CAA	CTT	TTT	ACG	TAG	AGT	GGG	GGT	CTT	CCA	CTA
Lys	Pro	Ser	Ile	Leu	Val	Glu	Lys	Cys	Ile	Ser	Pro	Pro	Glu	Gly	Asp>
1010	1020	1030	1040	1050											
*	*	*	*	*											
CCT	GAG	TCT	GCT	GTG	ACT	GAG	CTT	CAA	TGC	ATT	TGG	CAC	AAC	CTG	AGC
GGA	CTC	AGA	CGA	CAC	TGA	CTC	GAA	GTT	ACG	TAA	ACC	GTG	TTG	GAC	TCG
Pro	Glu	Ser	Ala	Val	Thr	Glu	Leu	Gln	Cys	Ile	Trp	His	Asn	Leu	Ser>
1060	1070	1080	1090	1100											
*	*	*	*	*											
TAC	ATG	AAG	TGT	TCT	TGG	CTC	CCT	GGA	AGG	AAT	ACC	AGT	CCC	GAC	ACT
ATG	TAC	TTC	ACA	AGA	ACC	GAG	GGA	CCT	TCC	TTA	TGG	TCA	GGG	CTG	TGA
Tyr	Met	Lys	Cys	Ser	Trp	Leu	Pro	Gly	Arg	Asn	Thr	Ser	Pro	Asp	Thr>
1110	1120	1130	1140	1150											
*	*	*	*	*											
AAC	TAT	ACT	CTC	TAC	TAT	TGG	CAC	AGA	AGC	CTG	GAA	AAA	ATT	CAT	CAA
TTG	ATA	TGA	GAG	ATG	ATA	ACC	GTG	TCT	TCG	GAC	CTT	TTT	TAA	GTA	GTT
Asn	Tyr	Thr	Leu	Tyr	Tyr	Trp	His	Arg	Ser	Leu	Glu	Lys	Ile	His	Gln>

Figure 31D

1160	1170	1180	1190	1200
*	*	*	*	*
TGT GAA AAC ATC TTT AGA GAA GGC CAA TAC TTT GGT TGT TCC TTT GAT	ACA CTT TTG TAG AAA TCT CTT CCG GTT ATG AAA CCA ACA AGG AAA CTA	Cys Glu Asn Ile Phe Arg Glu Gly Gln Tyr Phe Gly Cys Ser Phe Asp>		
1210 1220 1230 1240				
*	*	*	*	*
CTG ACC AAA GTG AAG GAT TCC AGT TTT GAA CAA CAC AGT GTC CAA ATA	GAC TGG TTT CAC TTC CTA AGG TCA AAA CTT GTG TCA CAG GTT TAT	Leu Thr Lys Val Lys Asp Ser Ser Phe Glu Gln His Ser Val Gln Ile>		
1250	1260	1270	1280	1290
*	*	*	*	*
ATG GTC AAG GAT AAT GCA GGA AAA ATT AAA CCA TCC TTC AAT ATA GTG	TAC CAG TTC CTA TTA CGT CCT TTT TAA TTT GGT AGG AAG TTA TAT CAC	Met Val Lys Asp Asn Ala Gly Lys Ile Lys Pro Ser Phe Asn Ile Val>		
1300 1310 1320 1330 1340				
*	*	*	*	*
CCT TTA ACT TCC CGT GTG AAA CCT GAT CCT CCA CAT ATT AAA AAC CTC	GGA AAT TGA AGG GCA CAC TTT GGA CTA GGA GGT GTA TAA TTT TTG GAG	Pro Leu Thr Ser Arg Val Lys Pro Asp Pro Pro His Ile Lys Asn Leu>		
1350	1360	1370	1380	1390
*	*	*	*	*
TCC TTC CAC AAT GAT GAC CTA TAT GTG CAA TGG GAG AAT CCA CAG AAT	AGG AAG GTG TTA CTA CTG GAT ATA CAC GTT ACC CTC TTA GGT GTC TTA	Ser Phe His Asn Asp Asp Leu Tyr Val Gln Trp Glu Asn Pro Gln Asn>		
1400 1410 1420 1430 1440				
*	*	*	*	*
TTT ATT AGC AGA TGC CTA TTT TAT GAA GTA GAA GTC AAT AAC AGC CAA	AAA TAA TCG TCT ACG GAT AAA ATA CTT CAT CTT CAG TTA TTG TCG GTT	Phe Ile Ser Arg Cys Leu Phe Tyr Glu Val Glu Val Asn Asn Ser Gln>		
1450 1460 1470 1480				
*	*	*	*	*
ACT GAG ACA CAT AAT GTT TTC TAC GTC CAA GAG GCT AAA TGT GAG AAT	TGA CTC TGT GTA TTA CAA AAG ATG CAG GTT CTC CGA TTT ACA CTC TTA	Thr Glu Thr His Asn Val Phe Tyr Val Gln Glu Ala Lys Cys Glu Asn>		
1490	1500	1510	1520	1530
*	*	*	*	*
CCA GAA TTT GAG AGA AAT GTG GAG AAT ACA TCT TGT TTC ATG GTC CCT	GGT CTT AAA CTC TCT TTA CAC CTC TTA TGT AGA ACA AAG TAC CAG GGA	Pro Glu Phe Glu Arg Asn Val Glu Asn Thr Ser Cys Phe Met Val Pro>		

Figure 31E

1540	1550	1560	1570	1580
*	*	*	*	*
GGT GTT CTT CCT GAT ACT TTG AAC ACA GTC AGA ATA AGA GTC AAA ACA CCA CAA GAA GGA CTA TGA AAC TTG TGT CAG TCT TAT TCT CAG TTT TGT Gly Val Leu Pro Asp Thr Leu Asn Thr Val Arg Ile Arg Val Lys Thr>				
1590	1600	1610	1620	1630
*	*	*	*	*
AAT AAG TTA TGC TAT GAG GAT GAC AAA CTC TGG AGT AAT TGG AGC CAA TTA TTC AAT ACG ATA CTC CTA CTG TTT GAG ACC TCA TTA ACC TCG GTT Asn Lys Leu Cys Tyr Glu Asp Asp Lys Leu Trp Ser Asn Trp Ser Gln>				
1640	1650	1660	1670	1680
*	*	*	*	*
GAA ATG AGT ATA GGT AAG AAG CGC AAT TCC ACA ACC GGA GAC AAA ACT CTT TAC TCA TAT CCA TTC TGC GCG TTA AGG TGT TGG CCT CTG TTT TGA Glu Met Ser Ile Gly Lys Lys Arg Asn Ser Thr Thr Gly Asp Lys Thr>				
1690	1700	1710	1720	
*	*	*	*	*
CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser>				
1730	1740	1750	1760	1770
*	*	*	*	*
GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg>				
1780	1790	1800	1810	1820
*	*	*	*	*
ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro>				
1830	1840	1850	1860	1870
*	*	*	*	*
GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala>				
1880	1890	1900	1910	1920
*	*	*	*	*
AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val>				

Figure 31F

1930	1940	1950	1960	
* * *	* * *	* * *	* * *	
AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr>				
1970	1980	1990	2000	2010
* * *	* * *	* * *	* * *	* * *
AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC TTT TGG Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr>				
2020	2030	2040	2050	2060
* * *	* * *	* * *	* * *	* * *
ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu>				
2070	2080	2090	2100	2110
* * *	* * *	* * *	* * *	* * *
CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys>				
2120	2130	2140	2150	2160
* * *	* * *	* * *	* * *	* * *
CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser>				
2170	2180	2190	2200	
* * *	* * *	* * *	* * *	
AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp>				
2210	2220	2230	2240	2250
* * *	* * *	* * *	* * *	* * *
TCC GAC GGC TCC TTC TTC CTC TAT AGC AAG CTC ACC GTG GAC AAG AGC AGG CTG CCG AGG AAG AAG GAG ATA TCG TTC GAG TGG CAC CTG TTC TCG Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser>				
2260	2270	2280	2290	2300
* * *	* * *	* * *	* * *	* * *
AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala>				

Figure 31G

2310 2320 2330 2340 2350
* * * * * * * * *
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA
GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys>

*
TGA
ACT
***>

Figure 32A

10	20	30	40	
*	*	*	*	
ATG GTG TGG CCG GCG CGG CTC TGC GGG CTG TGG GCG CTG CTG CTC TGC TAC CAC ACC GGC CGC GCC GAG ACG CCC GAC ACC CGC GAC GAC GAG ACG Met Val Trp Pro Ala Arg Leu Cys Gly Leu Trp Ala Leu Leu Cys>				
50	60	70	80	90
*	*	*	*	*
GCC GGC GGC GGG GGC GGG GGC GGC GGC CCT ACG GAA ACT CAG CGG CCG CCG CCC CCG CCG CCC CCG CGG CGC GGA TGC CTT TGA GTC Ala Gly Gly Gly Gly Gly Ala Ala Pro Thr Glu Thr Gln>				
100	110	120	130	140
*	*	*	*	*
CCA CCT GTG ACA AAT TTG AGT GTC TCT GTT GAA AAC CTC TGC ACA GTA GGT GGA CAC TGT TTA AAC TCA CAG AGA CAA CTT TTG GAG ACG TGT CAT Pro Pro Val Thr Asn Leu Ser Val Ser Val Glu Asn Leu Cys Thr Val>				
150	160	170	180	190
*	*	*	*	*
ATA TGG ACA TGG AAT CCA CCC GAG GGA GCC AGC TCA AAT TGT AGT CTA TAT ACC TGT ACC TTA GGT GGG CTC CCT CGG TCG AGT TTA ACA TCA GAT Ile Trp Thr Trp Asn Pro Pro Glu Gly Ala Ser Ser Asn Cys Ser Leu>				
200	210	220	230	240
*	*	*	*	*
TGG TAT TTT AGT CAT TTT GGC GAC AAA CAA GAT AAG AAA ATA GCT CCG ACC ATA AAA TCA GTA AAA CCG CTG TTT GTT CTA TTC TTT TAT CGA GGC Trp Tyr Phe Ser His Phe Gly Asp Lys Gln Asp Lys Lys Ile Ala Pro>				
250	260	270	280	
*	*	*	*	
GAA ACT CGT CGT TCA ATA GAA GTA CCC CTG AAT GAG AGG ATT TGT CTG CTT TGA GCA GCA AGT TAT CTT CAT GGG GAC TTA CTC TCC TAA ACA GAC Glu Thr Arg Arg Ser Ile Glu Val Pro Leu Asn Glu Arg Ile Cys Leu>				
290	300	310	320	330
*	*	*	*	*
CAA GTG GGG TCC CAG TGT AGC ACC AAT GAG AGT GAG AAG CCT AGC ATT GTT CAC CCC AGG GTC ACA TCG TGG TTA CTC TCA CTC TTC GGA TCG TAA Gln Val Gly Ser Gln Cys Ser Thr Asn Glu Ser Glu Lys Pro Ser Ile>				
340	350	360	370	380
*	*	*	*	*
TTG GTT GAA AAA TGC ATC TCA CCC CCA GAA GGT GAT CCT GAG TCT GCT AAC CAA CTT TTT ACG TAG AGT GGG GGT CTT CCA CTA GGA CTC AGA CGA Leu Val Glu Lys Cys Ile Ser Pro Pro Glu Gly Asp Pro Glu Ser Ala>				

Figure 32B

390	400	410	420	430
*	*	*	*	*
GTG ACT GAG CTT CAA TGC ATT TGG CAC AAC CTG AGC TAC ATG AAG TGT CAC TGA CTC GAA GTT ACG TAA ACC GTG TTG GAC TCG ATG TAC TTC ACA Val Thr Glu Leu Gln Cys Ile Trp His Asn Leu Ser Tyr Met Lys Cys>				
440	450	460	470	480
*	*	*	*	*
TCT TGG CTC CCT GGA AGG AAT ACC AGT CCC GAC ACT AAC TAT ACT CTC AGA ACC GAG GGA CCT TCC TTA TGG TCA GGG CTG TGA TTG ATA TGA GAG Ser Trp Leu Pro Gly Arg Asn Thr Ser Pro Asp Thr Asn Tyr Thr Leu>				
490	500	510	520	
*	*	*	*	*
TAC TAT TGG CAC AGA AGC CTG GAA AAA ATT CAT CAA TGT GAA AAC ATC ATG ATA ACC GTG TCT TCG GAC CTT TAA GTA GTT ACA CTT TTG TAG Tyr Tyr Trp His Arg Ser Leu Glu Lys Ile His Gln Cys Glu Asn Ile>				
530	540	550	560	570
*	*	*	*	*
TTT AGA GAA GGC CAA TAC TTT GGT TGT TCC TTT GAT CTG ACC AAA GTG AAA TCT CTT CCG GTT ATG AAA CCA ACA AGG AAA CTA GAC TGG TTT CAC Phe Arg Glu Gly Gln Tyr Phe Gly Cys Ser Phe Asp Leu Thr Lys Val>				
580	590	600	610	620
*	*	*	*	*
AAG GAT TCC AGT TTT GAA CAA CAC AGT GTC CAA ATA ATG GTC AAG GAT TTC CTA AGG TCA AAA CTT GTT GTG TCA CAG GTT TAT TAC CAG TTC CTA Lys Asp Ser Ser Phe Glu Gln His Ser Val Gln Ile Met Val Lys Asp>				
630	640	650	660	670
*	*	*	*	*
AAT GCA GGA AAA ATT AAA CCA TCC TTC AAT ATA GTG CCT TTA ACT TCC TTA CGT CCT TTT TAA TTT GGT AGG AAG TTA TAT CAC GGA AAT TGA AGG Asn Ala Gly Lys Ile Lys Pro Ser Phe Asn Ile Val Pro Leu Thr Ser>				
680	690	700	710	720
*	*	*	*	*
CGT GTG AAA CCT GAT CCT CCA CAT ATT AAA AAC CTC TCC TTC CAC AAT GCA CAC TTT GGA CTA GGA GGT GTA TAA TTT TTG GAG AGG AAG GTG TTA Arg Val Lys Pro Asp Pro His Ile Lys Asn Leu Ser Phe His Asn>				
730	740	750	760	
*	*	*	*	*
GAT GAC CTA TAT GTG CAA TGG GAG AAT CCA CAG AAT TTT ATT AGC AGA CTA CTG GAT ATA CAC GTT ACC CTC TTA GGT GTC TTA AAA TAA TCG TCT Asp Asp Leu Tyr Val Gln Trp Glu Asn Pro Gln Asn Phe Ile Ser Arg>				

Figure 32C

770 780 790 800 810
 * * * * * * * * * *
 TGC CTA TTT TAT GAA GTA GAA GTC AAT AAC AGC CAA ACT GAG ACA CAT
 ACG GAT AAA ATA CTT CAT CTT CAG TTA TTG TCG GTT TGA CTC TGT GTA
 Cys Leu Phe Tyr Glu Val Glu Val Asn Asn Ser Gln Thr Glu Thr His>

 820 830 840 850 860
 * * * * * * * * *
 AAT GTT TTC TAC GTC CAA GAG GCT AAA TGT GAG AAT CCA GAA TTT GAG
 TTA CAA AAG ATG CAG GTT CTC CGA TTT ACA CTC TTA GGT CTT AAA CTC
 Asn Val Phe Tyr Val Gln Glu Ala Lys Cys Glu Asn Pro Glu Phe Glu>

 870 880 890 900 910
 * * * * * * * * *
 AGA AAT GTG GAG AAT ACA TCT TGT TTC ATG GTC CCT GGT GTT CTT CCT
 TCT TTA CAC CTC TTA TGT AGA ACA AAG TAC CAG GGA CCA CAA GAA GGA
 Arg Asn Val Glu Asn Thr Ser Cys Phe Met Val Pro Gly Val Leu Pro>

 920 930 940 950 960
 * * * * * * * * *
 GAT ACT TTG AAC ACA GTC AGA ATA AGA GTC AAA ACA AAT AAG TTA TGC
 CTA TGA AAC TTG TGT CAG TCT TAT TCT CAG TTT TGT TTA TTC AAT ACG
 Asp Thr Leu Asn Thr Val Arg Ile Arg Val Lys Thr Asn Lys Leu Cys>

 970 980 990 1000
 * * * * * * * * *
 TAT GAG GAT GAC AAA CTC TGG AGT AAT TGG AGC CAA GAA ATG AGT ATA
 ATA CTC CTA CTG TTT GAG ACC TCA TTA ACC TCG GTT CTT TAC TCA TAT
 Tyr Glu Asp Asp Lys Leu Trp Ser Asn Trp Ser Gln Glu Met Ser Ile>

 1010 1020 1030 1040 1050
 * * * * * * * * *
 GGT AAG AAG CGC AAT TCC ACA GGC GCG CCT AGT GGT GGA GGT GGC CGG
 CCA TTC TTC GCG TTA AGG TGT CCG CGC GGA TCA CCA CCT CCA CCG GCC
 Gly Lys Lys Arg Asn Ser Thr Gly Ala Pro Ser Gly Gly Gly Arg>

 1060 1070 1080 1090 1100
 * * * * * * * * *
 CCC GCA AGC TCT GGG AAC ATG AAG GTC TTG CAG GAG CCC ACC TGC GTC
 GGG CGT TCG AGA CCC TTG TAC TTC CAG AAC GTC CTC GGG TGG ACG CAG
 Pro Ala Ser Ser Gly Asn Met Lys Val Leu Gln Glu Pro Thr Cys Val>

 1110 1120 1130 1140 1150
 * * * * * * * * *
 TCC GAC TAC ATG AGC ATC TCT ACT TGC GAG TGG AAG ATG AAT GGT CCC
 AGG CTG ATG TAC TCG TAG AGA TGA ACG CTC ACC TTC TAC TTA CCA GGG
 Ser Asp Tyr Met Ser Ile Ser Thr Cys Glu Trp Lys Met Asn Gly Pro>

Figure 32D

1160	1170	1180	1190	1200
*	*	*	*	*
ACC AAT TGC AGC ACC GAG CTC CGC CTG TTG TAC CAG CTG GTT TTT CTG	TGG TTA ACG TCG TGG CTC GAG GCG GAC AAC ATG GTC GAC CAA AAA GAC			
Thr Asn Cys Ser Thr Glu Leu Arg Leu Leu Tyr Gln Leu Val Phe Leu>				
1210 1220 1230 1240				
*	*	*	*	*
CTC TCC GAA GCC CAC ACG TGT ATC CCT GAG AAC AAC GGA GGC GCG GGG	GAG AGG CTT CGG GTG TGC ACA TAG GGA CTC TTG CCT CCG CGC CCC			
Leu Ser Glu Ala His Thr Cys Ile Pro Glu Asn Asn Gly Gly Ala Gly>				
1250 1260 1270 1280 1290				
*	*	*	*	*
TGC GTG TGC CAC CTG CTC ATG GAT GAC GTG GTC AGT GCG GAT AAC TAT	ACG CAC ACG GTG GAC GAG TAC CTA CTG CAC CAG TCA CGC CTA TTG ATA			
Cys Val Cys His Leu Leu Met Asp Asp Val Val Ser Ala Asp Asn Tyr>				
1300 1310 1320 1330 1340				
*	*	*	*	*
ACA CTG GAC CTG TGG GCT GGG CAG CAG CTG CTG TGG AAG GGC TCC TTC	TGT GAC CTG GAC ACC CGA CCC GTC GTC GAC GAC ACC TTC CCG AGG AAG			
Thr Leu Asp Leu Trp Ala Gly Gln Gln Leu Leu Trp Lys Gly Ser Phe>				
1350 1360 1370 1380 1390				
*	*	*	*	*
AAG CCC AGC GAG CAT GTG AAA CCC AGG GCC CCA GGA AAC CTG ACA GTT	TTC GGG TCG CTC GTA CAC TTT GGG TCC CGG GGT CCT TTG GAC TGT CAA			
Lys Pro Ser Glu His Val Lys Pro Arg Ala Pro Gly Asn Leu Thr Val>				
1400 1410 1420 1430 1440				
*	*	*	*	*
CAC ACC AAT GTC TCC GAC ACT CTG CTG CTG ACC TGG AGC AAC CCG TAT	GTG TGG TTA CAG AGG CTG TGA GAC GAC TGG ACC TCG TTG GGC ATA			
His Thr Asn Val Ser Asp Thr Leu Leu Leu Thr Trp Ser Asn Pro Tyr>				
1450 1460 1470 1480				
*	*	*	*	*
CCC CCT GAC AAT TAC CTG TAT AAT CAT CTC ACC TAT GCA GTC AAC ATT	GGG GGA CTG TTA ATG GAC ATA TTA GTA GAG TGG ATA CGT CAG TTG TAA			
Pro Pro Asp Asn Tyr Leu Tyr Asn His Leu Thr Tyr Ala Val Asn Ile>				
1490 1500 1510 1520 1530				
*	*	*	*	*
TGG AGT GAA AAC GAC CCG GCA GAT TTC AGA ATC TAT AAC GTG ACC TAC	ACC TCA CTT TTG CTG GGC CGT CTA AAG TCT TAG ATA TTG CAC TGG ATG			
Trp Ser Glu Asn Asp Pro Ala Asp Phe Arg Ile Tyr Asn Val Thr Tyr>				

Figure 32E

1540	1550	1560	1570	1580
*	*	*	*	*
CTA GAA CCC TCC CTC CGC ATC GCA GCC AGC ACC CTG AAG TCT GGG ATT				
GAT CTT GGG AGG GAG GCG TAG CGT CGG TCG TGG GAC TTC AGA CCC TAA				
Leu Glu Pro Ser Leu Arg Ile Ala Ala Ser Thr Leu Lys Ser Gly Ile>				
*				
1590	1600	1610	1620	1630
*	*	*	*	*
TCC TAC AGG GCA CGG GTG AGG GCC TGG GCT CAG TGC TAT AAC ACC ACC				
AGG ATG TCC CGT GCC CAC TCC CGG ACC CGA GTC ACG ATA TTG TGG TGG				
Ser Tyr Arg Ala Arg Val Arg Ala Trp Ala Gln Cys Tyr Asn Thr Thr>				
*				
1640	1650	1660	1670	1680
*	*	*	*	*
TGG AGT GAG TGG AGC CCC AGC ACC AAG TGG CAC AAC TCC TAC AGG GAG				
ACC TCA CTC ACC TCG GGG TCG TGG TTC ACC GTG TTG AGG ATG TCC CTC				
Trp Ser Glu Trp Ser Pro Ser Thr Lys Trp His Asn Ser Tyr Arg Glu>				
*				
1690	1700	1710	1720	
*	*	*	*	
CCC TTC GAG CAG TCC GGA GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA				
GGG AAG CTC GTC AGG CCT CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT				
Pro Phe Glu Gln Ser Gly Asp Lys Thr His Thr Cys Pro Pro Cys Pro>				
*				
1730	1740	1750	1760	1770
*	*	*	*	*
GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA				
CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT				
Ala Pro Glu Leu Leu Gly Pro Ser Val Phe Leu Phe Pro Pro Lys>				
*				
1780	1790	1800	1810	1820
*	*	*	*	*
CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG				
GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC				
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val>				
*				
1830	1840	1850	1860	1870
*	*	*	*	*
GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC				
CAC CAC CTG CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG				
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr>				
*				
1880	1890	1900	1910	1920
*	*	*	*	*
GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG				
CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC GGC GCC CTC CTC				
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu>				

Figure 32F

1930	1940	1950	1960	
*	*	*	*	*
CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC				
GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG				
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His>				
 1970	1980	1990	2000	2010
*	*	*	*	*
CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA				
GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG CAG AGG TTG TTT				
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys>				
 2020	2030	2040	2050	2060
*	*	*	*	*
GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG				
CGG GAG GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC				
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln>				
 2070	2080	2090	2100	2110
*	*	*	*	*
CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG				
GGG GCT CTT GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC CTC CTC TAC				
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met>				
 2120	2130	2140	2150	2160
*	*	*	*	*
ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC				
TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG AAG ATA GGG				
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro>				
 2170	2180	2190	2200	
*	*	*	*	*
AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC				
TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG				
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn>				
 2210	2220	2230	2240	2250
*	*	*	*	*
TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC				
ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG				
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu>				
 2260	2270	2280	2290	2300
*	*	*	*	*
TAT AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC				
ATA TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG				
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val>				

Figure 32G

2310 2320 2330 2340 2350
* * * * * * * * * *
TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG
AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln>

2360 2370 2380
* * * * * *
AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA
TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
Lys Ser Leu Ser Leu Ser Pro Gly Lys ***>

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter that is claimed and for which a patent is sought on the invention entitled RECEPTOR BASED ANTAGONISTS, AND METHODS OF MAKING AND USING, the specification of which is attached hereto.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to in the oath or declaration.

I acknowledge the duty to disclose information of which I am aware that is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States Application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) that occurred between the filing date of the prior application and the national or PCT international filing date of this application:

USSN 60/101,858 Filed September 25, 1998

And I hereby appoint Robert J. Cobert (Registration No. 36,108), Gail M. Kempler (Registration No. 32,143), and S. Leslie Misrock (Registration No. 18,872) and each of them my attorneys, each with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected

✓
Att. Docket No. REG 203-A
Declaration and Power of Attorney
Page 2

therewith and to file any International Applications that are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone calls, regarding this application to:

Robert J. Cobert, Esq.
Regeneron Pharmaceuticals, Inc.
777 Old Saw Mill River Road
Tarrytown, New York 10591
Tel. (914-345-7400)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Inventor: NEIL STAHL

Signature: _____

Date: _____

Citizenship: United States of America

Residence: RD #10, Kent Shore Drive

Carmel, New York 10512

United States of America

Post Office Address: same as residence

Inventor: GEORGE D. YANCOPOULOS

Signature: _____

Date: _____

Citizenship: United States of America

Residence: 1519 Baptist Church Road

Yorktown Heights, New York 10598

United States of America

Post Office Address: same as residence